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RECENT ADVANCES
IN
PATHOLOGY

RECENT ADVANCES IN PATHOLOGY

SIXTH EDITION

By Various Authors

Edited by

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PREFACE TO THE SIXTH EDITION

During the preparation of the last edition of this book my former co-author, Professor L. P. Garrod, became deeply involved in the many administrative and research problems connected with the chemotherapy of infection and, as it was clearly impossible for him to relinquish his increasing responsibilities in this field, a happy collaboration extending over sixteen years came to a regrettable end.

In this edition I have had the good fortune to enlist the help of a small panel of contributors who, in carrying out their share in the production of the book, have made my task as general editor a relatively light one, and writing this preface provides the opportunity for me to thank them for their willing co-operation and exemplary patience.

In a review of the first edition of "Recent Advances in Pathology," written twenty years ago, it was pointed out that "the authors in the Recent Advances series, not being under any cramping necessity to give a full account of their subject . . . write about what has interested them and the reader gets the benefit." This generalization may apply with greater force to this than to previous editions, as each contributor has written about that part of pathology in which he has taken a personal interest and has special experience.

One of the functions of a general editor is to eliminate needless overlapping and tiresome repetition and, whilst some elimination has been necessary, I have not exercised my privilege when the expression of two different views on the same debatable problem appeared to be either instructive or interesting.

The general plan of the book follows that of previous editions, but whenever opportunity permitted a deliberate attempt has been made in this edition to discuss disorders of function and their correlation with general physiological principles. The chapter on renal disease, for example, is wholly concerned with pathological states in which there is gross functional abnormality of the tubular apparatus and the biochemical consequences of this are specially stressed. The account of pulmonary disease covers a wider field than in previous editions. The section on the endocrine organs has been completely re-written in the form of a

general review of the whole system, and whilst this has necessitated a certain amount of omission, it is hoped that the separate accounts of each of its component parts will link up with one another, and thereby imitate in some small measure the integration which is so striking a characteristic of the functional activity of this group of organs.

Finally, as on five other similar occasions, it is my very pleasant duty to thank the publishers for their invaluable help and great forbearance.

G. H.

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CHAPTER I

INFLAMMATION

SINCE the publication of Metchnikoff's many contributions to comparative pathology towards the end of the last century, pathologists have come more and more to regard the inflammatory reaction in higher animals as a complex defensive response which has evolved under the pressure of Natural Selection. Once this point of view had been widely accepted, it was inevitable that the former purely objective study of the phenomena of inflammation should be followed by efforts to interpret the various phases of the process in the light of their possible survival value to an animal subjected to infection or injury. In adopting such a quasi-teleological attitude, the pathologist need offer no apology. Though as a biologist he can no longer concede " design " in Nature, he is prepared to admit a " purpose," if by the term " a purposive response " is meant a sequence of reactions that renders more likely the survival of the individual and species. If the succeeding account of recent work on the phenomena and significance of inflammation seems on first consideration to be undesirably coloured by this outlook, it can be advanced in favour of this Darwinian version of teleology that it has not only often provided an intellectually satisfying explanation for many complicated processes in living structures, but that it has also proved notably fruitful in suggesting ideas and hypotheses which have directed the way to further discoveries. For this latter reason alone, the concept of " purpose " must be accepted as a powerful creative influence in the modern development of the biological sciences.

The Vascular Reactions in Injured Tissues

The Mechanism and Significance of the Hyperæmia. For more than a century it has been known that small blood vessels in the vicinity of injured tissues undergo widespread dilatation in the early stages of inflammation. With the advent of Immunology, the significance of these vascular responses appeared in a new light, for pathologists at once inferred that the local hyperæmia occupied a central place in the whole reaction because it enabled

the threatened area to be supplied with the phagocytes and antibodies on whose presence a successful defence was believed to depend. In recent years, experiments have been carried out on several lines which give further support to this inference.

How important, even critical, this prompt local vasodilatation may prove is shown by experiments in which the widening of the arterioles—which is probably the chief factor in causing the active hyperæmia—is prevented by the injection of adrenalin into an infected site in the incipient stages of inflammation. Rigdon (1940) injected four areas in the skin of a rabbit with a culture of *Staphylococcus aureus*, and then re-injected two of these areas at two-hourly intervals subsequently with saline and two with a dilute solution of adrenalin. The skin at the sites injected with bacteria and saline became hyperæmic and swollen in six hours, while that in the areas injected with bacteria and adrenalin showed neither change. Histological sections from these lesions, however, showed large numbers of neutrophil leucocytes surrounding and ingesting the bacteria in the saline-injected sites, but practically no immigration of leucocytes in the areas in which the hyperæmia had been suppressed by the adrenalin.

Recently these observations have been much extended by Evans, Miles and Niven (1948). Saline suspensions of a number of species of pathogenic bacteria, with and without the addition of 2μ gm. of adrenalin, were injected both intradermally and intramuscularly into guinea-pigs. The detrimental effect of the adrenalin was very evident when the anaerobic clostridia, *Cl. welchii* and *Cl. septicum*, were inoculated ; with the addition of this drug, the number of bacilli needed to establish a fatal infection was reduced to less than one-thousandth of that required in the presence of saline only. A similar, though not so marked, enhancement of pathogenic action in the presence of adrenalin was also found with several strains of staphylococci and streptococci. Since similar tests made with some of the toxic filtrates of the bacterial species used showed that adrenalin exerted little additive effect to their potency, it seems clear that this drug acts by facilitating the *infection* and not the *intoxication*. Whatever deleterious effect the adrenalin may have is almost certainly restricted to the first hour or two after the infection has taken place, for the skin-blanching action of the drug administered in this dose and manner only persists for this period of time. During this critical interval, however, few leucocytes and scarcely any exudate collected in the adrenalin-infiltrated area.

The inhibitory effect of adrenalin upon exudate formation can also be readily demonstrated with vital dyes. If an area of active hyperæmia is produced in the skin of a normal animal by the injection of a small dose of histamine or of certain bacterial toxins, a vital dye such as pontamine blue or trypan blue when injected intravenously escapes through the walls of the locally more permeable capillaries and stains the affected area blue. Should the site be infiltrated at the same time with adrenalin, however, none of the dye escapes and the area remains uncoloured.

It seems, therefore, that adrenalin ischæmia interferes with the supply of oxygen, plasma and leucocytes to the affected area, so that local anoxia develops and both the formation of the exudate and the emigration of the leucocytes are equally minimized. In the absence of exudation, antibacterial substances do not accumulate in the area, while the paucity of neutrophils and macrophages reduces the extent of phagocytosis to well below the critical level needed for successful local defence.

The effectiveness of the experimental induction of acute temporary vaso-constriction in promoting infection with pathogenic anaerobic bacteria has a practical parallel in the occurrence of gas gangrene in patients after the intramuscular injection of various therapeutic substances, one of the commonest of which seems to be adrenalin. Cooper (1946) has reported a typical instance in which the injection of adrenalin into a man's arm, for the suppression of some allergic manifestation, was promptly followed by clinical gas gangrene, and drew attention to the frequency with which similar sequelæ have been recorded in the Continental literature.

The clear separation of nervous from chemical components in the inception of active hyperæmia that was made by Lewis (1927) from his study of the effects of injury in the skin, has led to much speculation as to the nature of the chemical agent or agents concerned. The belief, at first tentatively advanced by Lewis, and later more confidently by Dale (1929), that histamine plays the major chemical vaso-dilator *rôle* has since received much support from other investigators. It has not, however, passed without criticism. That histamine inoculated into the skin can bring about many of the vascular phenomena found in acute inflammation has been abundantly demonstrated; what is needed, however, to convert an assumption based mainly on analogy into an established fact is the proof by properly controlled pharmacological tests that this particular substance can

be recovered in abnormally large amounts from injured tissues. This proof has been provided by Kellaway and his colleagues (1947), who have made a long and detailed study of the pharmacologically-active substances that are liberated from tissues damaged by heat, various forms of radiant energy, animal venins, bacterial toxins and other noxious agents. In all the instances in which such injured organs were perfused, these investigators were able to identify histamine in the outflowing fluid.

An informative experiment on similar lines, though on a more restricted scale, was carried out by Rosenthal and Minard (1939). Taking very thin surface shavings from human skin and from that of some common laboratory animals, they arranged the film of living epidermis over the open end of a glass tube in such a way that its external aspect could be irritated by the application of various physical or chemical agents, while its inner deeper layers were in contact with saline ; by this device any soluble products from the injured tissue would diffuse into the fluid in the tube. Tests made upon such fluids after the film of epidermis had been burned or electrically stimulated, showed that a substance had entered them which resembled histamine in the following important respects : it caused the contraction of guinea-pig's intestines ; it was dialysable and heat stable ; it was destroyed by histaminase ; and its actions were inhibited by anti-histamine drugs. These authors, therefore, concluded that Lewis's H-substance was really histamine, and that the pain caused by injuries to the skin is partly or wholly attributable to the action of this liberated substance on nearby pain nerve-endings.

While the *rôle* of histamine as a natural participant in the phenomena of acute inflammation now seems assured, it appears unlikely that it is the sole tissue metabolite concerned in evoking such a complex series of reactions. Indeed, as will be seen, its release cannot account for the chemotaxis of the neutrophils. In recent years, therefore, increasing attention has been directed to other substances, and especially to adenine compounds, both as pharmacologically-active products of tissue injury and as possible effectors of some of the vascular responses that are typical of the ensuing inflammation. Adenosine triphosphate has been found by Folkow and his co-workers (1948) among others to be a potent vaso-dilator drug, and its local release at the site of injury may well contribute both to the active hyperæmia and to the movements of the neutrophils. The possible biological actions of the adenine nucleotides in shock and other pathological

conditions involving tissue destruction have recently been reviewed by Green and Stoner (1950).

The Changes in Vascular Permeability in Acute Inflammation. It has long been known that exudates have a higher concentration of protein than transudates (a point that is sometimes of diagnostic value), and much evidence has been collected to show that this results from an increase in the permeability of the capillary endothelium in the immediate vicinity of the affected focus. In recent years, the detection of such an increase in permeability has been facilitated by the use of certain dyes, such as trypan blue, which on intravenous injection become firmly attached to plasma albumin, and consequently cause a distinctive coloration of the tissues should the dye-protein complex escape into the tissue spaces. This device was first used for this purpose by Ramsdell (1928), who regarded the accumulation of the dye in an area as an indicator of injury to the local capillary endothelium. This interpretation has since received general support ; several striking coloured illustrations of such selective escape of dyes in inflamed sites are to be found in Burrow's (1932) monograph.

The nature of the chemical agent that causes increased capillary permeability in inflammation has been much discussed. Largely under the influence of Lewis and Dale, most of the earlier workers attributed this change also to the liberation of histamine in the injured area. Rocha e Silva and Dragsted (1941), who support this view, injected a pure solution of histamine into the skin in quantities comparable to those that they found by pharmacological assay in extracts of damaged tissues, and found that this substance gave the same trypan blue permeability response as the tissue extracts themselves. In spite of these and other similar findings, however, a case has been made by several investigators, of whom the most active has been Menkin (1940), for the occurrence in inflammatory exudates of other chemical agents capable of effecting these characteristic alterations in capillary permeability. To what he regarded as the principal agent, he gave the name "*leukotaxine*," a term which he selected, for a reason that will be mentioned later, because he believed that it also favoured the chemotaxis of neutrophils in inflamed areas. Menkin has attempted the chemical separation of this agent from the many other substances present in inflammatory exudates, and has described the most potent of his extractives as a thermostable, moderately diffusible, nitrogenous compound that is probably a

polypeptide that has been liberated during the active proteolysis known to take place in exudates. Some of Menkin's observations and conclusions have since been corroborated by Cullumbine and Rydon (1946) and by Spector (1951), who agree in believing that the most active component of the exudate is a polypeptide of low molecular weight which is probably released by the action of proteolytic enzymes on damaged tissues. Both Menkin and Cullumbine (1947) draw attention to several believed differences between leukotaxine and histamine ; amongst them is the statement that the former substance produces a diffuse blue patch at the site of inoculation in trypan blue injected animals, while the latter only causes a peripheral blue ring. It has been pointed out, however, by Bier and Rocha e Silva (1938) that in his experiments Menkin injected very large quantities of histamine into the skin —much larger amounts than are ever likely to be liberated under any natural conditions in injured tissues. At these artificially high concentrations, they maintain, histamine produces a blue ring, but at lower concentrations the coloration is typically diffuse (see Rocha e Silva (1946) for a coloured illustration showing this difference). The widely differing amounts of histamine used may well account for some if not all of the features of the reaction described by Menkin and Cullumbine on the one side, and Rocha e Silva and his colleagues on the other. If so, it would seem that Rocha e Silva's interpretation is to be preferred so far as the participation of histamine in inflammation is concerned.

Whatever constituent of the exudate is responsible for enhancing local capillary permeability, and there may very probably be more than one involved, the escape of plasma proteins into the tissue fluid of the affected area exercises an important influence on the course of the ensuing inflammation. In individuals who are experiencing a particular infection for the first time, the mere accumulation of the so-called "*natural antibodies*," as well as the physical dilution of the toxic metabolites of the bacteria, is of considerable defensive value, while in those who have already acquired some immunity, either actively or passively, the important contributions made by specific antibodies are added. Should foreign antibacterial or antitoxic serum be administered for prophylactic or therapeutic purposes, they also tend to become concentrated at site of acute inflammation by the same mechanism of altered capillary permeability. During the recent war, however, Miles and Miles (1943) drew attention to an important corollary to this general proposition, in that any elevation in pressure of the

tissue fluids in infected areas will retard the escape of both homologous and heterologous antibodies. As long as the inflamed tissues remain lax, the hydrostatic pressure of the tissue fluids offers a negligible resistance to the escape of the exudate through the capillary endothelium, but once the local pressure has risen appreciably—a phenomenon which can often be observed, for example, in the increasing tenseness of the skin round a large boil—the outward passage of further fluid from the small blood-vessels is more strongly opposed. For this reason, amongst others, specific therapeutic sera are likely to manifest their greatest effect if given early in the course of the infection ; when administered later, they fail to saturate the site of bacterial activity and are of less avail in the *local* defence processes accordingly.

An interesting recent application of the phenomenon of heightened capillary permeability in infected foci, which may in time prove of clinical diagnostic value, is the locating of sites of acute inflammation by the aid of proteins that have been marked with some radioactive element. Trypan blue can readily be rendered radioactive by the insertion of the isotope of bromine, Br^{82} (which has the short half-life of thirty-four hours) into the dye during the process of its synthesis. After injection into the blood, this nearly related compound behaves in the same way as ordinary trypan blue ; it becomes coupled to the plasma albumin and consequently escapes, together with this protein, into any acutely inflamed focus. With the help of a directional Geiger-Muller counter, Moore and Tobin (1942) were able to locate concentrations of this radioactive dye in an abscess which had been produced experimentally in rabbits with *Staphylococcus aureus*. The method clearly has important practical possibilities for comparable localization studies in man.

The Spread of Bacteria from Infected Areas

The Local Dispersal of Bacteria at Sites of Initial Infection. Different species of pathogenic bacteria present very wide variations in the rapidity with which they spread in the tissues from their primary site of invasion. Since the sequels of such dissemination, sometimes culminating in a generalized bacteriæmia, may be so serious, it is desirable in the interests of rational treatment to acquire a full knowledge of those factors that are likely to promote or retard the process in its critical early stages.

When small numbers of pathogenic bacteria are inoculated with a minimum of trauma into the tissues of a normal animal, it is found that within a few hours they become dispersed from the small focus at which they were initially deposited. During experiments on these lines, in which he used pneumococci because of the ease with which they could be recognized histologically, Rich (1933) confirmed the observations of several earlier investigators who had found that in normal unimmunized animals the inoculated bacteria " drifted " progressively farther and farther from their site of injection. With non-motile organisms such as the pneumococcus, this dispersal must be a passive one, and can only be brought about by movements in the surrounding tissue fluids. Any attempt to understand the initial stages of a local infection should therefore begin with a study of those forces which create movements and currents in the fluids in the tissue spaces.

Some of the local factors concerned in causing the constant ebb and flow of extravascular fluids in normal and inflamed tissues have been carefully studied by McMaster and his associates, and have been discussed by them in an important series of papers in the *Journal of Experimental Medicine* between 1938 and 1947. Most of these investigations have been carried out by direct microscopical observations on the spreading of minute quantities of a vital dye that had been injected into living tissues with great care to avoid unnecessary trauma to the surrounding structures. One of the major facts which has emerged from these studies is the importance of constant pulsation in the tissues for preventing the stagnation of extravascular fluid in the intercellular spaces and of lymph in the lymphatics. In experiments on the living amputated ears of rabbits, it was found (McMaster and Parsons, 1938 ; Parsons and McMaster, 1938) that the rate of centripetal spread of a vital dye was profoundly affected by the mode of perfusion of the nutrient blood. Perfusion through the central artery at a constant pressure caused the fluid to pass through the walls of the smaller blood-vessels in a manner that accorded with Starling's theory, but instead of any excess entering lymphatics and flowing towards the base of the ear, it collected in the tissue spaces and rendered the connective tissues increasingly œdematous. When the flow of perfusing blood was made intermittent—in a pulsatile manner resembling that produced by the heart beats in a normal animal—by using a pump to deliver it at pressures alternating from 140 mm. Hg systolic to 60 mm. diastolic, the formation of œdema was prevented or delayed. With this

vascular rhythm, moreover, the wider dispersal of the tissue fluid at the site of injection of the dye, and the more rapid centripetal flow of lymph could be readily followed from the local changes in colour. In the microcosm of soft living tissues, every element is in a state of constant agitation, the result of the moment-by-moment impact of the cardiac pulse upon the small arterial vessels, and this rhythmic movement is transmitted to all their surrounding structures and the tissue fluids between them.

In addition to the rapid oscillatory movements imparted to the tissue fluids by the pulse, there are others imposed by forces which act irregularly but often powerfully. The first and most obvious of these is that produced by muscular contractions, both of the skeletal muscles and the smooth muscle of the viscera. Such movements bring about great rises in tissue pressure, and operate importantly in the return of venous blood and lymph from the periphery to the heart. Their significance for bacterial dissemination was fully appreciated by the older surgeons, who followed Hilton (1863) in strictly enjoining rest for infected structures. The second of these irregular causes of movement of tissue fluid is less evident ; according to McMaster (1941), it is probably dependent upon phasic dilatations and constrictions of small arteries of the kind described by Krogh (1929). Such alterations in arteriolar resistance necessarily entail corresponding local differences in capillary pressure, so that any small family of capillaries may at one time be the seat of excessive transudation and at another of reabsorption. This implies no contravention of Starling's theory, which was devised to apply to the average in-and-out movements of fluid for many small vessels in macroscopic volumes of tissue. On the minute scale of dimensions commensurate with the sizes of micro-organisms, such averages no longer apply, and should bacteria be present they become carried to and fro in the tissues by the irregular ebb and flow of the local tissue fluid. To those interested in the mechanics of a spreading infection, such considerations as those discussed by McMaster are of fundamental importance.

In recent years, two further factors that may possibly be concerned in the local dispersal of bacteria have been much discussed. The first is the enzyme hyaluronidase, which is known to be liberated by many species of bacteria and to be capable of hydrolyzing hyaluronic acid, a substance which in the polymerized state forms one of the important cement substances of the tissues. This enzyme is nearly related to the " spreading factor " recovered

from certain animal tissues, notably the testis (Duran-Reynals, 1942). In spite of the *a priori* likelihood that hyaluronidase would promote the extension of infection with any bacterium capable of forming it, through loss of cohesion between the tissue elements, it has proved difficult to demonstrate by convincing experiments that it actually exerts any material effect on the course of an infection. Indeed, such evidence as has yet been collected seems to indicate that there is little correlation between hyaluronidase formation and invasiveness. Evans (1943) determined the lethal dose for guinea-pigs for three strains of *Cl. Welchii*, of which two only formed hyaluronidase, and found no significant difference between them all. Nor did he find that the administration of a serum containing a considerable concentration of the immune body, anti-hyaluronidase, had any beneficial effect on the progress of an infection with one of the strains that formed this enzyme freely. Similarly, Crowley (1944), in a study of some of the biological features of over 300 strains of Group A hæmolytic streptococci that had been recovered from human beings, found no evidence to suggest that a capacity to form hyaluronidase was related to the virulence of the bacterium. Selbie and Simon (1952), working with 75 strains of *Staphylococcus pyogenes*, also failed to find any correlation between hyaluronidase production and virulence. For the present, therefore, the possible *rôle* of this enzyme in promoting the spread of bacteria must be regarded as unproven.

The second of these debated questions has been the possible importance of the fibrin which is commonly laid down about a focus of acute inflammation as a physical obstacle that confines the enclosed bacteria. Menkin (1940), who has applied the term "*fibrin barrier*" to this encapsulation, has attributed great importance to its localizing action. He regards the mesh-work of fibrin, with its openings only one or two micra wide, as a fine sieve which occupies not only the local tissue fluid spaces, but also the lumina of the lymphatics that lead from the area. Rich (1936), on the other hand, is unwilling to accept this essentially mechanical explanation for the localization of the bacteria during the incipient stages of an inflammatory reaction. He draws attention to the accelerated lymph flow from inflamed tissues—a feature that is difficult to reconcile with any widespread obstruction to lymphatics—and believes instead that much of the so-called "*fixation*" results from the agglutinative properties of natural antibodies for the micro-organisms present. Menkin, while

prepared to admit the effectiveness of agglutinins in already immunized animals, cannot agree with Rich as regards the importance of their contribution to the localization of invasive bacteria in normal animals that are experiencing the infection for the first time. Along these lines, he has attempted the reconciliation of these apparently divergent views. In any discussion upon the dispersal or fixation of micro-organisms at an infected site, however, it must not be forgotten that inflammation is a dynamic process, and consequently that the degree of localization of such particulate matter as bacteria may vary according to the stage of the response that has been reached. That such variations during the evolution of the reaction do occur is indicated by the observations of Barer (1952), who found that the rapidity of spread of the radio-opaque material "Thorotrast" from inflamed tissues is much affected by the nature, severity and stage of development of the lesion. The whole question is fully reviewed by Hadfield and Garrod (1945).

The Lymphatics as Pathways for the Dispersal of Bacteria. Surgeons have long been familiar with the bright red streaks that sometimes appear on the limbs of persons suffering from acute pyogenic infections of the hands or feet and are known as "*acute lymphangitis*." These lines, which extend sometimes as far as the regional lymph node, are due to the centripetal passage of lymph containing toxic materials and even bacteria from the focus of infection, and the consequent inflammatory dilatation of the smaller blood-vessels around the lymphatic trunk involved. They have been reproduced experimentally by Lewis by injecting small amounts of histamine intradermally; the drug enters the local lymphatic trunk, but being very diffusible some of it escapes from the returning lymph into nearby tissues and causes the surrounding capillaries at once to dilate. Much light on the whole question of lymphatic participation in acute inflammation has been thrown by the experimental studies of Drinker and his associates in recent years; on account of their close bearing on various practical problems arising in the treatment of infectious foci, they will be briefly reviewed.

Many experiments made by Drinker and his collaborators (Drinker and Yoffey, 1941) have shown how greatly the centripetal flow of lymph is increased both by acute inflammation and by vigorous active or passive movements of the part concerned. By inserting a cannula in a major lymphatic trunk draining the lower part of an anæsthetized dog's leg, it was possible to determine

the changes in pressure, rate of flow and protein concentration of the outflowing lymph after a mild thermal burn of the foot (Field, Drinker and White, 1932). When the temperature of the water applied to the skin reached 60° C., the venous pressure in the afferent veins rose suddenly—a change that could be attributed to the onset of arteriolar dilatation and widespread active hyperæmia. At the same time, the lymph pressure too rose sharply, and fluid began to pour freely from the cannula. The pressure of the lymph might reach 10 mm. Hg, and its protein concentration might rise, because of the greater permeability of the affected blood capillaries, from its normal level of about 1·5 per cent. to three or four times that value. That this excessive flow of lymph is not necessarily a temporary feature of the inflammatory reaction is shown by the finding that in one dog which was maintained under anæsthesia for twenty-four hours the lymph was still escaping spontaneously at the end of this period. Were the acute inflammation to be of infective origin instead of a sterile one caused by heat, such an accelerated lymph flow must bear with it the toxic products of the pathogenic bacteria and possibly some of the bacteria themselves.

The second major cause for an accelerated centrally-directed flow of lymph is active or passive exercise of the part concerned. Drinker and Field (1933) examined the effect of movement by cannulating a lymphatic trunk in an anæsthetized dog just below the knee, and attaching the leg to a device which alternately flexed and extended it about once a second—roughly the rate of a walking movement. Soon after exercise began, the pressure in the lymph trunk rose steadily, and the centripetal flow of lymph, which at rest had been negligible, increased correspondingly. With active as well as with passive exercise, acceleration of lymph drainage takes place, so that the return of lymph, like that of blood, appears to depend on the massaging effect of alternations in pressure in the intermuscular spaces in which such vessels lie. Although no such quantitative estimates of the effect of activity have been made for man, there is clinical evidence for the belief that undue exercise of an infected limb has a detrimental action on the spread of any infection. Attention has already been drawn to Hilton's advocacy of rest for an infected part ; the same line of treatment has recently been brought again to the fore by Trueta (1946), who immobilizes infected injured limbs by encasing them in plaster.

In one part at least of its centripetal course, the flowing lymph

has to pass through the tortuous sinuses of a lymph node, where it comes into contact with cells of the reticulo-endothelial system under conditions very favourable for phagocytosis. Long ago, Field, Drinker and Ward (1934) showed how effectively this percolation through a node rid the lymph of any bacteria carried with it. Large numbers of streptococci perfused into a lymph-node by way of its main afferent trunk, were removed from the fluid, so that what emerged was almost or quite sterile. Filtration became less effective, however, if the perfusion rate was increased—a finding very relevant to the above discussion on the influence of muscular movement on the rate of lymph flow. The fate of bacteria once inside the lymph node, and the type of cellular reaction that they excite, has recently been re-studied by Smith and Wood (1949). They found that in rats, whose hind feet had been inoculated intradermally with small volumes of fluid cultures of pneumococci or streptococci, the bacteria could be seen in the cortical sinus of the regional node within five minutes. From this time onwards the signs of acute lymphadenitis increased, and the blood capillaries and lymph sinuses inside the node became increasingly dilated. Neutrophil leucocytes accumulated in the lymph sinuses, some of them arriving by way of afferent lymphatic trunks and some migrating from local blood capillaries. Phagocytosis of the bacteria by neutrophils took place with great rapidity, so that after a few hours few extracellular bacteria could be seen. Thenceforward, the neutrophils increasingly fell victims to the bacteria ; the infection was terminated, however, when these early defensive cells, together with their ingested organisms, were in turn phagocyted and digested by the large macrophages that line the nodal sinuses. Throughout the height of the infection the lymph nodes were enlarged and doubtless tender, if not painful ; the experiments reproduced closely the acute lymphadenitis which is frequently associated with some regional suppurative lesion in man.

Before ending this short review of some of the general principles that apply to the spread of bacteria along lymphatics, it will be of interest to refer briefly to a few diseases of man, in whose pathogenesis this mode of extension of infection is known to be important. In tuberculosis the rapid involvement of regional lymph nodes has long been recognized to be an almost invariable accompaniment of any *primary* infection, either in the lungs or intestines. Together with any lesions at the portal of entry, such as a Ghon focus in the lungs, it forms what Ranke described as

the "*primary complex*" (Kayne, Pagel and O'Shaughnessy, 1948). The experiments of Freund and Angevine (1938) indicated that this lymphatic spread occurs early, for they found that after a small intradermal inoculation of *M. tuberculosis*, the bacilli could be recovered from regional nodes twenty-four to forty-eight hours later. In syphilis there have been few demonstrations of the pathways taken by the spirochætes in dispersing from their portal of entry at the primary chancre. Zurhelle (1921), however, has thrown some light on the question by making biopsies on inguinal lymph nodes from patients during the primary and secondary stages of the disease. He found numerous spirochætes in such nodes, some apparently alive, but many that had been ingested by macrophages, and he expressed the belief that the reactive changes that developed in the lymph nodes were essentially defensive. So commonly are spirochætes now known to be present in such regional nodes that examination by dark-ground illumination of biopsy puncture fluid from them has become a recognized method in the diagnosis of early syphilis (Stokes *et al.*, 1944). In some forms of streptococcal infection, notably in erysipelas, the bacteria spread freely by lymphatics and can often be recognized in such distended vessels. Again, the condition has been closely reproduced experimentally: Angevine (1936) injected virulent hæmolytic streptococci intradermally in rabbits, and traced their progressive dispersal along lymphatics until, about an hour later, they had reached the regional nodes. How successful was the defence set up by this structure was shown by the comparatively small numbers of streptococci recoverable from tissues along their efferent lymphatics. In lymphogranuloma venereum (Stannus, 1933), in bubonic plague (Report, 1907; Wyssokowitsch and Zabolotny, 1897) and in tularæmia (Forbus, 1943), the regional inguinal or axillary lymph nodes are rapidly and severely involved. So slight is the lesion at the portal of entry in the two former diseases that it often escapes recognition and the lymph nodal enlargement is clinically the presenting feature.

The Behaviour of Leucocytes in Acutely Inflamed Tissues

The Agents responsible for Chemotactic Movements. The approach of leucocytes to a focus of injury is one of the most significant of all the cellular responses in inflamed tissues, because it is largely on its effectiveness that the subsequent phagocytic destruction of any invading micro-organisms and the eradication of an infection ultimately depends. The literature on this impor-

tant subject is therefore extensive, and has been fully reviewed by McCutcheon (1942, 1946); here it is only necessary to refer to a few of the more important recent observations, and to relate them to already accepted views.

Shortly after the pioneer work on the chemotaxis of motile plant cells, when the migration of leucocytes in the neighbourhood of bacteria was first studied, it was believed that the substances responsible for determining their forward or backward movements emanated mainly, if not wholly, from the micro-organisms themselves. It has been clearly shown by many *in vitro* studies, in which living organisms and their chemical extractives have been used, that many species of pathogenic and non-pathogenic bacteria can produce substances that have this action, and thereby create diffusion gradients in their surroundings. The most satisfactory of such experiments, less liable to an equivocal interpretation than many of the earlier ones, are those of McCutcheon and Dixon (1936). A simple method was devised, which they and others have used extensively since, for recording the minute by minute movements of individual leucocytes in the vicinity of small clusters of bacteria. By keeping preparations containing the white cells warm and moist, and plotting the path followed by each leucocyte with the help of a camera lucida, they were able to show how sensitively the neutrophils respond to the presence of nearby bacteria. With many species of micro-organisms, such a plotted chart of the track of approach of the leucocytes was almost rectilinear.

Although with the great majority of the bacterial species studied chemotaxis has been found to be positive, that is, the leucocyte advances towards the bacteria, a few observations have been made in which the opposite has been observed. From quite early in the experimental analysis of infective processes, it has been often suggested that the virulence of certain bacteria is connected with their ability to repel leucocytes. Many of these conclusions were undoubtedly based on evidence that might equally well be used to support alternative explanations. In recent years, however, examples of negative chemotaxis of an unquestionable kind have been described. Stevenson and Reed (1940) studied the chemotaxis exerted by thirteen strains of *Staphylococcus aureus* for explanted neutrophils, and found a notable correlation between the pathogenicity of the different strains and their repulsion of the leucocytes. McCutcheon *et al.* (1939) described the behaviour of neutrophils in the vicinity of

streptococci, first the advance of the white cell and later its precipitate retreat. An interesting feature of these experiments, however, was the observation that on those occasions in which the leucocyte and streptococcus chanced to come into contact, the latter were actively phagocyted. These two aspects of leucocyte behaviour towards micro-organisms—chemotaxis and phagocytosis—thus seem to be independently conditioned phenomena.

The application of tissue culture and other *in vitro* methods to the study of chemotaxis has made it possible to distinguish between the different degrees of sensitivity displayed by the different types of leucocytes. In general, it can be stated that the neutrophils are much the most responsive to the stimuli provided by most of the common pathogenic bacteria, and partly for this reason and partly because of their more rapid rate of locomotion, they form the great bulk of immigrant cells in suppurative foci. Monocytes, the representatives of the reticulo-endothelial system in the circulating blood, were found by Coman (1940) to be little attracted by either staphylococci or tubercle bacilli, to both of which neutrophils showed pronounced positive chemotaxis, and similar findings have been recorded by Lasfargues and Delaunay (1947). In spite of the apparent absence of bacterial chemotaxis for monocytes, however, these cells gravitate, though more slowly than neutrophils, to infected foci; it may well prove, should the experiment be made, that monocytes are more motivated by products of injured tissues than by substances liberated by bacteria.

Although belief in the importance of bacterial metabolites as chemotactic agents for neutrophils has in no way lessened in recent years, greater interest has tended to centre on the participation of diffusible products released by the damaged tissues themselves. When it first appeared likely that histamine was responsible for the vaso-dilatation after injury, repeated attempts were made to determine if in addition to this action it possessed chemotactic attraction for leucocytes. Grant and Wood (1928) examined this possibility with negative results, however, and a number of other investigators have since confirmed their findings. Although histamine itself does not seem to be concerned in the process, it none the less seems probable that some products of tissue injury possess such an attraction, for it is well known that neutrophils accumulate in large numbers round masses of sterile necrosed tissues such as cardiac infarcts and sterile thermal burns.

For example, in some experiments in which mild burns were inflicted on human skin by placing a test tube of hot water briefly in contact with it, Moon (1935) was able to count the leucocytes in the resulting bleb fluid, and found that their numbers rose rapidly during the first few hours. Since at this time the fluid itself was sterile, no other conclusion can be drawn than that whatever substance attracted the leucocytes was derived from the injured tissues themselves.

A number of attempts have been made to isolate factors from injured tissues and exudates which can induce such a leucocyte migration. Menkin (1940) has pursued this line of investigation actively, and has succeeded in recovering from exudates a crystalline material which, as stated above, he has termed " *leukotaxine* " on account of this chemotactic property. The more important of Menkin's observations have since been confirmed by Cullumbine and Rydon (1946), who found that a substance with these properties could be obtained after thermal and chemical burns, and agree with Menkin in regarding the active agent as a soluble, diffusible, polypeptide that is released after proteolytic digestion of the tissue proteins. That such a breakdown of protein can take place in the skin after an injury has been shown by Beloff and Peters (1945) in their study on burns. It seems likely, therefore, that after any acute injury to tissues, whether by a living or non-living agent, or even by acute anoxia, substances of the general nature of leukotaxine are released. It is the presence of these substances which excites the chemotaxis of the neutrophils and increases the permeability of the local capillaries.

In addition to the polypeptides of the types included under leukotaxine, it seems likely that adenine compounds, released through the breakdown on nucleoproteins in the injured tissues, may also exert a positive chemotactic effect on neutrophils. Bennet and Drury (1931) first demonstrated that some of the compounds of this purine base possess such an action, when they found that the instillation of a saline solution of adenylic acid into the conjunctival sac of a rabbit's eye was followed both by local vasodilatation and the accumulation of neutrophil pus in the inner canthus. It has long been known, moreover, that compounds of adenylic acid, now frequently used in the form of pentanucleotides in the treatment of agranulocytosis (Goodman and Gilman, 1941), exert a strongly stimulant action on leucopoiesis in the bone marrow. The release of these substances from injured tissues may thus be responsible for both the local

accumulation of neutrophils at sites of tissue injury and the general leucocytosis that accompanies many infections.

The Phagocytosis of Bacteria. The mechanics of phagocytosis and the factors that may affect its efficiency have recently been comprehensively reviewed by Berry and Spies (1949). It is inevitable, as well as obviously desirable, that much of the recent work on this aspect of the inflammatory reaction has been directed to seeing in what ways this natural defensive process can be beneficially affected through immunological and chemotherapeutic advances. Such studies will pave the way to further progress in our means of supplementing the natural defences of the body against infections, for although in most instances the phagocytosis of micro-organisms is followed by their intracellular destruction, experiments have shown that some virulent bacteria may survive ingestion and in time kill the engulfing leucocyte (Rogers, *et al.*, 1952 ; Suter, 1952). In such infections, and perhaps most notably in tuberculosis, the principle of using potent but relatively insoluble particulate antibiotics, which like the micro-organisms themselves are ingested by the phagocytes, may lead to results of practical value by fortifying the cellular defences at this critical point (see Markham, *et al.*, 1951, a, b, c, ; Florey, 1952 ; Heatley, *et al.*, 1952).

Soon after the introduction of the sulphonamides by Domagk, the suggestion was made that these substances owed much of their efficacy to their stimulation of phagocytosis by neutrophil leucocytes. To-day the main bulk of evidence seems to be against this view, and to support the belief that any increase in the phagocytosis of bacteria that occurs in the presence of these drugs depends upon their interference in the metabolism of the micro-organisms, and consequently with the release of noxious substances by them. This latter point of view has been clearly expressed by Domagk (1947), who has summarized his standpoint in the description of the following experiment. If a mouse is inoculated intramuscularly with a virulent hæmolytic streptococcus, no localizing inflammatory reaction develops, but if such an infected animal receives a sulphonamide, innumerable leucocytes enter the area and destroy the bacteria. The previously virulent micro-organisms now become as readily phagocyted as ordinary avirulent saprophytes, a result which follows from the injury inflicted by the drug upon the streptococci and not from any stimulus it might afford to the leucocytes. Very similar conclusions were reached by Lushbaugh and Cannon (1942) after they had made a com-

parative study of the sequelæ of experimental subcutaneous infections with virulent pneumococci in sulphonamide treated and untreated rabbits. In untreated animals the bacteria multiplied freely with little opposition from phagocytic leucocytes, until eventually the infection became generalized. On the other hand, in the rabbits which received sulphapyridine, no comparable growth of cocci took place, and any bacteria that were present were rapidly disposed of by immigrant neutrophils.

The proof that the sulphonamides operate primarily by injuring susceptible bacteria is rendered still clearer in an ingenious experiment made by Harris and Miller (1941). A small quantity of a virulent strain of a hæmolytic streptococcus was introduced into and sealed inside a small collodion sac, which was then inserted into the peritoneal cavity of a rabbit. In an animal that had received sulphanilamide, the bacteria within the sac died out completely, *though neither antibody proteins nor leucocytes were able to enter it*, the pores of the sac being too small. In an untreated rabbit, the streptococci multiplied many hundred thousandfold during the course of the experiment. Such findings show quite clearly that these chemotherapeutic agents act very powerfully on such virulent yet susceptible bacteria as the hæmolytic streptococcus, and may under favourable circumstances bring about their destruction without the aid of the cellular defences of the tissues. The conditions imposed in such an experiment as this, however, are necessarily artificial, and under any ordinary circumstances of infection, in which the access of the leucocytes is not impeded, there is little doubt that the course of defence follows the lines indicated above by Domagk.

There is clear evidence that some species of bacteria can form metabolites which are highly destructive to leucocytes. These substances, which are known under the general term "*leucocidin*," are well-known toxins formed by virulent strains of staphylococci, streptococci and some of the clostridia, and form a powerful weapon in the armament of these bacteria. Lasfargues and Delaunay (1946) have made an interesting study of the toxic action of some of these bacterial products on the cells of splenic implants in tissue cultures, and have described the highly lethal effects of filtrates from cultures of staphylococci and *Cl. Welchii*, and the less potent, though still considerable, activities of those from diphtheria and typhoid bacilli. Fortunately, by immunotherapeutic measures the effects of these noxious substances can often be effectively countered, and the vital defensive powers

of the leucocytes preserved. In the first place, the severity of the injury that pathogenic bacteria inflict on the tissues and their supporting leucocytes is closely dependent upon the local concentrations attained by their toxic products. Any bacteriostatic substance, whether a sulphonamide, an antibiotic or an immune body, will thus tend by restricting the multiplication of the organisms to moderate the local damage. Bacteria, like all living beings, tend to multiply in geometrical series, and any agent which can lengthen the interval between successive bacterial divisions has a far-reaching effect on the final population reached, and hence on the ultimate concentration of toxic products. In the second place, many of these bacterial products are antigenic, and their effects can therefore often be neutralized by administration of the appropriate specific antiserum. The value of the aid that such immune bodies are sometimes capable of providing was admirably shown in an experiment made by Stewart (1943) on the effect of antitoxins in gas gangrene. To one-half of a group of guinea-pigs infected intraperitoneally with *Cl. Welchii*, a dose of the corresponding antitoxic serum was given. Films made from the peritoneal exudates that formed in the two groups of animals showed a striking difference ; those from the unprotected guinea-pigs showed multiplying bacilli, dying leucocytes and hardly any phagocytosis, while those from the antitoxin group showed fewer bacilli, healthy leucocytes and frequent ingestion of micro-organisms. The former animals all died, while the latter survived. The essential difference between these two groups was that the neutralization of the toxin by the passively administered antitoxin made it possible for the cellular defence mechanism of the host to operate to advantage.

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CHAPTER II

HYPERSENSITIVITY REACTIONS

It is a well-recognized finding in Clinical Medicine that certain persons react sharply and severely to exposure to some foreign substance, for example, pollens, animal dandruffs, antitoxic sera, foods, industrial chemicals, penicillin, sulpha drugs, and some bacterial products such as tuberculin, which for most individuals appear to be quite innocuous. It seems that with such persons, for reasons which will be discussed below, some particular tissue or tissues have acquired an abnormal sensitivity to the specific agent, so that a degree of contact which with normal people produces no recognizable tissue disturbance, now results in an often unexpected and wholly incommensurate injury. In short, for such individuals an ordinarily harmless material has for some reason acquired a highly noxious character. Although idiosyncrasies of this kind—of which "hay-fever" is a notable instance—had long been recognized, their importance first became widely appreciated in the final decade of the last century when antitoxins produced in horses were introduced for the treatment of diphtheria and tetanus. The frequency with which an injection of these therapeutically potent sera was followed by distressing and sometimes fatal sequelæ led to many investigations into the causation of anaphylaxis and "serum sickness," and eventually to a clear understanding of their underlying immunological mechanisms. It was this elucidation of the basic nature of these reactions to foreign sera that provided the clues to the pathogenesis of other clinical manifestations of the "hypersensitivity state." The careful correlation of clinical and laboratory studies has now led to the almost general acceptance of the view that, although their symptoms are very diverse and their provocative agents almost innumerable, asthma, many food idiosyncrasies, some forms of contact dermatitis and other "allergic" [1] diseases,

[1] The term "allergy," from the Greek ἄλλος, ἔργον meaning an altered capacity to react (Veränderten Reaktionsfähigkeit), was coined by von Pirquet (1906). Its definition as finally propounded by him was so wide that it is now desirable to use it in a narrower sense as applying to an altered capacity to react in which there is evidence for some underlying immunological mechanism. Since such a change may take the form of either an exaggerated or a diminished reactivity, the term allergy is clearly more inclusive than hypersensitivity for it would cover immunity as well. Because the former are generally more conspicuous in pathology than the latter, however, the terms "hypersensitivity" and "allergy" are now used almost interchangeably. The brevity and euphony of the latter word are also recommendations for its use.

also depend upon some common mechanism of an essentially immunological character.

There is little doubt that hypersensitivity is mainly an acquired state that develops as a result of exposure to some environmental agent. But before discussing the condition from this standpoint, it will be well to refer briefly to the possibility that an hereditary element enters significantly into its ætiology, if only because Coca and his associates (1931), who have been very prominent in this field of medicine, have attributed such importance to genetical factors in its causation as to justify, in their opinion, the separation of many allergic diseases into a special category to which they have given the name "atopy." While formerly, mainly through the influence of this school, the importance of heredity was overstressed, latterly, because of the steady encroachments made on the concept of atopy by discoveries of fresh immunological mechanisms in previously obscure hypersensitive states, it has received less attention than its importance probably deserves.

The present position regarding heredity as an ætiological factor in allergy may perhaps be summarized as follows. Presumptive evidence in favour of some genetical element comes from the large number of family histories in which cases of allergic manifestations, notably asthma and migraine, recur with a frequency that is difficult to reconcile with chance. Such a familial incidence remains, nevertheless, compatible with some exclusively environmental ætiology, and it could only be after rigorous treatment of available pedigrees by modern genetical methods that this latter possibility could be excluded. For several reasons, unfortunately, this statistical treatment is difficult to apply. Firstly, even the strongest proponents of the importance of heredity now regard the abnormal genetical constitution merely as a predisposition which can only lead to clinical manifestations if the necessary environmental factors are given the opportunity to become operative. For this reason alone, the incidence of clinical cases in any pedigree may fall short to an unknown extent of those genetically affected. Secondly, even in those states that are generally accepted as allergic, there may be wide variations in the age at which the hypersensitivity may declare itself by symptoms or signs, so that again some potentially allergic members of the family may be overlooked. For these two reasons alone, any recorded pedigrees must necessarily be incomplete. Lastly, the group of diseases that has been attributed to allergy is mani-

fold, and with some of them no general agreement on their allergic nature has been reached. With these and other difficulties to contend with, it is hardly surprising that professional geneticists have been discouraged from exploring the very ill-defined territory of the allergic diseases. The evidence in favour of an inheritable element of significant potency is thus no more than *prima facie*, and only deserves the credence that can be placed on testimony of that kind. (For a fuller discussion, see Cockayne, 1933; Neel, 1947.)

The Immunological Basis of Hypersensitivity

Before entering on a discussion of the various functional and structural disturbances associated with allergic reactions, it will prove helpful to review briefly some of the concepts that are necessary for an understanding of their pathogenesis in the light of present-day immunology.

1. The early experiments of anaphylaxis and hypersensitivity were all carried out with foreign proteins, often with horse serum, whose antigenic powers could readily be demonstrated both in man and in laboratory animals. While these findings soon provided an explanation for a number of forms of hypersensitivity met with in clinical practice, they failed to account for the many instances in which repeated exposures to certain non-protein substances, often of a simple chemical constitution, were followed by allergic manifestations. Classical amongst such substances are the dyes ursol (*p*-phenylenediamine) and aurantia (hexanitrodiphenylamine), used for staining furs and leather, and latterly some of the sulpha drugs used in treating infections (see Longcope, 1943; Davis, 1949; Hawking and Lawrence, 1950). The pioneer work of Landsteiner and his associates (Landsteiner, 1945) on "synthetic" antigens has provided the links needed to connect these two forms of hypersensitivity. These investigators found that some of the chemicals which commonly evoke allergic reactions in the persons who handle them prove to be highly reactive with proteins under conditions of temperature and H ion concentration found in living tissues. Consequently, when they come into contact with epidermal or other tissue proteins, modified proteins are formed whose "foreign" nature can be recognized by the cells of the individual, and whose absorption into lymph or blood results in the creation of specific antibodies. It has thus become apparent that most, if not all, substances that evoke hypersensitivity do so either because they are themselves foreign

proteins (as are most antisera) or by reason of their ability to combine as " haptenes " (Landsteiner) or " pro-antigens " (Gell, Harington and Rivers, 1946) with the individual's own proteins (as are some dyes and drugs), and in that way impart the character of foreignness to these formerly normal tissue constituents. In either case, the entry of the abnormal protein is followed by the production of the corresponding antibody. Landsteiner's investigations have thus brought about a unification of a number of forms of hypersensitivity—especially those whose main clinical manifestations are in the skin—along the lines of classical immunology.

2. It is now known that the modified globulins which are produced in the tissues under the influence of an antigenic stimulus, and which are collectively known as specific antibody, are not all necessarily of the same molecular configuration (see Marrack, 1951). Those formed in horses early in the course of diphtheria immunization, for example, fall into the gamma fraction, while those produced later separate in the beta fraction of the globulins. Not only may these early and late antibodies differ from one another physico-chemically, but they may also differ significantly in their immunological behaviour (see Topley and Wilson, 1946 a). It is well known to producers of commercial antitoxins, for instance, that sera from the early bleedings of a horse undergoing immunization often lack the " avidity," or speed and firmness of combination with toxin, that generally characterizes the sera obtained subsequently (see Barr, 1951). When the antigen is a synthetic one produced by the conjugation of a reactive chemical with an homologous protein, or even more than one homologous protein, the likelihood of heterogeneity in the resulting antibody is still greater, as Harington and his associates have pointed out. This increased diversity of the antibody molecules formed is a reflection of the inevitable gradation in the foreignness of the antigenic complex. If only one molecule of the chemical combines with one of the protein, for instance, the homologous character of the protein will be hardly affected, but if fifty of the former combine with one of the latter, as may easily happen if the concentration of the chemical is high, a wholly foreign antigenic conjugate will be formed (see Haurowitz *et al.*, 1941). Since in allergy due to the simpler chemicals the concentration of the dye or drug may vary widely in different parts of the exposed tissues, pro-antigen-protein complexes with very varying ratios may be produced simultaneously, and the antibodies formed will possess a corre-

spondingly wide range in their powers of combining with them. At first appearance, this point might seem academic, but it may well prove that this broad spectrum of specificity, extending to varying grades of foreignness in the antigenic complexes, has particular significance in that form of allergy known as "contact dermatitis."

It now seems likely that this heterogeneity of behaviour of monospecific antibodies extends also to a further important attribute—their tendency to persist in the free state in the blood and tissue fluids as "circulating" antibody, or to become in some way incorporated into the surface membranes of particular cells as "fixed" or "sessile" antibody. *The distinction between the two, which has been drawn mainly from experiments on anaphylaxis, is fundamental for an understanding of current views on the pathogenesis of hypersensitivity.* Much evidence supports the belief that whereas combination of antigen and antibody can take place without any detrimental effect on the individual as long as both are free in the blood or tissue fluids, any union that occurs when either has become fixed is followed by serious disturbances in the cells in whose membrane or interior the process happens. Clearly, any antigen introduced parenterally will combine preferentially with the most accessible antibody, namely, that in the blood and tissue fluids; should the latter be present in substantial excess, the individual would be termed immune. If the circulating antibody is proportionately deficient, however, the entering antigen will unite with the more remote sessile antibody, and an injury to the affected cells results; such a person is in the hypersensitive state. Immunity and hypersensitivity are thus both dependent on the action of a provocative antigen—the essential difference between them lies in the amount and distribution of the specific immune bodies as between body fluids and cells.

This distinction between immunity and hypersensitivity may be made clearer by considering a concrete example—a comparison of the principles that underlie the well-known Schick and Mantoux tests. In diphtheria, tissue injury is brought about by a protein product of the bacteria which is both toxic and antigenic, and in this latter capacity it can cause the formation of a large amount of the specific antitoxin. In a recently immunized person, most if not all of this persists as circulating antibody in the blood and tissue fluids, in both of which its presence can readily be demonstrated by protection experiments on animals. In tuberculosis

the bacillary antigens excite the production of much less circulating antibody, a feature which is clearly shown by the low titres for precipitins and complement-fixing antibodies in the sera of most patients suffering from the disease. What antibody is formed, moreover, appears to become preferentially attached to cells as sessile antibody (see p. 47). In a person who has recently been successfully immunized against diphtheria, therefore, a re-inoculation of the specific exotoxin, as in the Schick test, results in no recognizable tissue injury—the negative reaction—because the whole dose of toxin becomes neutralized by the free antibody of the tissue fluids before it can attack the nearby cells. In a person who has been infected with the tubercle bacillus, on the other hand, any of the specific protein antigen that may be inoculated into the tissues (such as the P.P.D. in the Mantoux test) fails to become neutralized by the insignificant amount of free antibody in the tissue fluids, and is consequently at liberty to combine with the sessile antibody of neighbouring cells. It is the resulting injury to these cells which calls forth the erythema typical of the positive tuberculin test. For this reason, such materials as tuberculo-proteins, which are devoid of any "primary toxicity," are conveniently described by Gay's term—"toxallergens."

If the specific antigen is brought into relation with the sessile antibody slowly and in a dilute form, the latter may become saturated without any explosive reaction, and for the time the tissues become "desensitized." Once such desensitization has occurred, the antigen—such as a foreign serum—may be introduced rapidly without fear of dangerous results, and this impunity continues as long as the desensitized state persists. After a few weeks, however, the antigen which has brought about the temporary desensitization is lost, and in the ensuing months the former state of hypersensitivity gradually returns. These basic principles should be borne in mind whenever foreign sera, such as antitoxins are to be given to persons who, by preliminary skin tests, have been found to possess specific hypersensitivity to these animal proteins.

3. From his pioneer experiments on anaphylaxis, Richet concluded that the combination of an antigen with its antibody led to the release of a poisonous substance to which he gave the name "anaphylatoxin." Dale's illuminating experiments on the reactions of isolated organs—notably the uterus—from sensitized animals have been chiefly responsible for rendering this view untenable, and substituting for it the concept of reactions

with sessile immune bodies on cell surfaces. When a uterus from a sensitized animal is suspended in an organ bath to which an excess of antibody has been added, no contraction follows the introduction of the specific antigen, though both immune bodies unite in the surrounding fluid under conditions which are eminently suited for the demonstration of anaphylatoxin should any be formed. Such experimental conditions have their human counterpart in a negative Schick test. On the other hand, if such a sensitized uterus, taken from an animal which had been well perfused with saline to free the tissues from circulating antibody, is suspended in a bath and specific antigen added, a contraction occurs promptly—the diffusion of the antigen into the substance of the organ leads to its union with the sessile antibody situated on the surfaces of the muscle cells. This mutual neutralization is injurious to the cells involved, and leads to the local release of substances which are potent vaso-motor drugs, as in the acute inflammatory response to noxious physical and chemical agents. This latter type of isolated organ reaction clearly has close analogies with the Mantoux tuberculin test.

There are several reasons for regarding histamine as the most important of these products of injury in sensitized organs: the close resemblance of the signs of anaphylactic shock and acute histamine poisoning (Dale, 1950); the mutual inhibition of skin reactions with histamine and with antigens in sensitized persons (Hare, 1926); the demonstration of an increased histamine content in shocked organs (Schild, 1939); and the lessening of the severity of anaphylactic shock phenomena by anti-histamine drugs (Feinberg, 1947). Whether acetylcholine is similarly liberated is doubtful. Farber and his colleagues (1944) were unable to recover it from shocked organs even after the addition of eserine to lessen the likelihood of its destruction by cholinesterase. Much still remains to be explored in the pharmacology of anaphylactic shock, and it is possible that such active compounds as adenosine, acetylcholine, heparin, histamine, lysolecithin and potassium salts may be released by different organs under these conditions, as Kellaway and his colleagues (see Kellaway, 1947) found to happen in intoxications with the toxins of some of the gas gangrene producing clostridia.

In broad and partly simplified terms, the present-day concept of hypersensitivity may be outlined as follows. Primary sensitization depends on the entry into the tissues either of some undenatured foreign protein or of some simpler but reactive

chemical which, by its combination with the individual's own proteins, can confer the character of foreignness upon the latter. Such an exposure, which may be either single or repeated, leads to the formation by the reticulo-endothelial system of specific antibody, much of which becomes distributed throughout the body by way of the circulation. On entering the tissue fluids, some of this antibody becomes incorporated in the membranes of certain types of cell, notably smooth muscle and epidermal cells, and there remains, perhaps for many years, as sessile antibody. In time, the titre of the circulating antibody evoked by this primary sensitization falls to an insignificant level, so that should a further exposure occur, there is no protective circulating antibody in the blood to combine with the freshly incoming antigen and so prevent it from reaching and uniting with the sessile antibody of vulnerable cells. Serious functional disturbances are then likely to follow ; partly from the injury inflicted on the cells immediately involved, and partly from the release of pharmacologically active products of cell damage—notably histamine, and possibly adenosine and acetylcholine. In fatal anaphylactic shock in susceptible animals which have undergone a general sensitization, and in which injuries due to this antigen-antibody combination occur in many different tissues simultaneously, the clinical signs are mainly those of acute histamine poisoning. On the other hand, if the sessile antibody is chiefly localized in one structure, such as the skin or the bronchi, the manifestations will be correspondingly distinctive. The clinical picture in allergy will depend to only a minor extent on the nature of the particular antigen, for they all act as toxallergens, not as primary toxins. On the other hand, the picture will be mainly determined by two factors : firstly, the route—parenteral injection, skin contact, inhalation or ingestion—by which the specific antigen re-enters the tissues, and secondly on the distribution of the cells that bear the specific sessile antibody amongst the different organs.

The Ways in which Hypersensitivity Develops

It has already been pointed out that although hereditary factors possibly affect the readiness with which any particular person may develop hypersensitivity, the state is essentially an acquired one that finds its origin in a former exposure to some antigenic protein or potential haptene. How and when the antigen gained access to the tissues may not always be determinable, but

sufficient knowledge of the manifold forms of clinical allergy has now been gained for it to be clear that the sensitizing agent can enter the body in a number of different ways. The following paragraphs direct attention to some of the more common and important routes and modes of entry. For fuller treatment of these and other aspects of hypersensitivity, various works on Allergy should be consulted (Ratner, 1943 ; Vaughan and Black, 1948 ; Urbach, 1946 ; and Rackemann's " Annual Reviews " in the *Archives of Internal Medicine*).

Parenteral Injection. The hypersensitivity which follows the injection of unrefined foreign antitoxic serum was the earliest form of allergy to be studied systematically by immunological methods and still remains the prototype for other forms. The lengthy and intensive study of anaphylaxis and serum sickness has provided most of the basic knowledge of the pathogenesis of the hypersensitive state. In recent years, the problems of allergy have been enlarged and complicated by the steadily increasing use of parenteral methods for the therapeutic administration of drugs. Arsphenamine compounds, various antibiotics such as penicillin, general and local anæsthetics, insulin, liver extracts and other potent drugs of animal origin are now commonly given in single or repeated injections directly into the blood or tissues, and many instances of a resulting hypersensitivity have been recorded.

Ingestion. From the studies of Schloss and Worthen (1916), Walzer and Walzer (1935) and others, it is now known that small quantities of undenatured food proteins may be absorbed in an immunologically active state through the intestinal mucosa. The entry of such unsplit proteins by this route seems to occur with special ease in very young infants, whose intestinal epithelium is more permeable to these large molecules then than later. But even in older persons, especially if the mucosa is for any reason inflamed, permeability again increases and allows the entry of large antigenic molecules (Gutzeit, 1932). Fish, egg, milk and some fruit proteins are particularly liable to excite hypersensitivity in this way.

Were any doubt to remain that these food allergies are basically immunological in nature, it should be dispelled by Prausnitz and Küstner's (1921) discovery that hypersensitivity to particular proteins can be passively conferred on a small area of skin in a normal person—a finding that led later to the development of their well-known test. In this reaction, which in principle is

analogous to experimental passive anaphylaxis in animals, a small volume (0·1 ml.) of serum from the supposedly sensitive person is injected intracutaneously in the skin of the forearm of a normal subject, and so creates an area of sensitization which persists for many days. Some hours must be allowed to elapse to give the transferred antibodies time to become attached as sessile antibodies to the skin cells of the recipient. If the test is positive, a trace of the specific protein similarly inoculated into the area twenty-four hours later evokes within a few minutes a typical "triple response" of Lewis. This reaction which, like the histamine test which it closely resembles, consists of erythema, urticaria and flare, and persists for about an hour before it fades.

In addition to the ingestion allergy associated with the absorption of unsplit proteins, certain drugs given orally sometimes lead to specific hypersensitivity. Iodides and the sulpha drugs are notable for the occurrence of these troublesome side reactions, the former combining with the amino-acid tyrosine in the serum proteins to form iodo-proteins (Wormall, 1930) and the latter becoming attached to serum albumin in some way not yet determined (Davis, 1949). The sensitizing therapeutic substances of low molecular weight are as a class reactive compounds, and probably form "foreign" proteins in the tissues after their absorption from the intestines, though in some instances such antigenic material may be produced by bacterial activity in the lumen of the gut and absorbed in an already complex form.

Inhalation. Hypersensitivity of the respiratory tract, in the clinical forms of hay fever or asthma, is a common form of allergy; it can be caused in a variety of ways, chief amongst which is the inhalation of certain kinds of organic dusts (see Rimington *et al.*, 1947). The pollens of certain grasses and the dandruff of horses and other common domestic animals have been recognized for more than a century as provocative agents of these distressing respiratory disorders. In recent years, the number of such recognized sensitizing agents has been much increased through the introduction of many new organic materials into commerce and industry. In the Lancashire textile industry the well-known disability which begins as "Monday Fever," but later extends to most of the working week, is the result, as Prausnitz (1936) has shown, of the periodic re-exposure to protein-containing dust from the cotton. The dusts from certain varieties of tropical timbers introduced into this country for cabinet making have also been found to provoke asthma in some of the workmen

(Doig, 1949). Although the number of persons disabled by any single form of industrial sensitization is usually small, the wide variety of the occupational respiratory sensitizations gives interest and importance to this growing group of allergic diseases.

There is no longer any doubt that sensitization of the respiratory tract can arise from the absorption of antigenic material contained in dust that is inhaled and deposited on the mucosa of the bronchi. The question has been approached experimentally by Ratner and Gruehl (1929), who found that guinea-pigs placed in an insufflation chamber into which dried horse dandruff or castor bean meal dust were atomized, developed a sensitivity that led to immediate respiratory anaphylaxis on further exposure to the same material some weeks later. These observations have since been confirmed and extended to other antigens by several investigators (see Urbach *et al.*, 1947).

Skin Contact. A large and growing number of chemicals widely used in industry and domestic life are capable of evoking hypersensitivity through their interaction with the epidermis and their consequent conferment of antigenic powers on its constituent proteins. Various dyes, notably ursol and aurantia, can cause serious eczemas, both in the makers and users of fur and leather goods (see Schwartz *et al.*, 1947). The compound "tetryl," which was used in explosive manufacture during the recent war, caused an alarming incidence of allergic dermatitis amongst the girls who handled it (Gell, 1944), while formalin and phenylhydrazine both have a sinister reputation in this respect amongst laboratory workers who use them frequently. This latter chemical is so potent as a toxallergen that instances have been recorded of chemists who have developed a generalized eczematous eruption after a colleague had used it in the same room. The sensitizations with members of the genus *Primula* are well known to gardeners, while on the American continent poison oak and poison ivy are common causes of allergic dermatitis for the unwary hiker.

Whether these sensitizing agents ordinarily act through uninjured skin, or whether they require some superficial abrasion before they can penetrate, is not known and would require to be determined independently for each. The matter is of little practical significance, however, for with most persons, and especially those engaged in manual labour, small traumas of the hands are commonplace. That skin lesions can facilitate the entry of some allergens, however, was clearly shown by Cranston Low (1924) and by Bloch (1929), who found that even minor

excoriations, such as those created by lightly drawing a piece of sandpaper over the skin, greatly added to the sensitizing action of an extract of primula. Any persistent moist state of the skin also seems to promote the sensitizing power of dyes and other chemicals, perhaps through the local loosening of the horny layer of the epidermis. On such areas, too, bacterial and fungal populations are likely to flourish, and in some cases these organisms may effect some metabolic transformation that converts an initially innocuous substance into an active allergen.

Infections. In several infectious diseases, of which tuberculosis is outstandingly the most important, there are grounds for regarding allergy as playing a highly significant part, both in the pathogenesis and progress of the disease. Tuberculous animals, and by inference human beings also, acquire an easily demonstrated hypersensitivity to the protein products of the tubercle bacillus within a few weeks of the primary infection. With re-infection, the accelerated and intensified inflammatory reaction at the site of entry of the second inoculum of bacteria was originally described by Robert Koch, and has since been known as "Koch's phenomenon." How far this pronounced allergic response contributes to the protection of individuals rendered hypersensitive by some prior successfully-resisted infection has been much disputed, and still remains an unsettled question. The usually trivial allergic response to intracutaneously inoculated tuberculin, however, has provided in the form of the Mantoux test a simple and reliable indicator of past or present tuberculous infection in man and domestic animals which has proved particularly valuable in epidemiological and epizootical surveys.

With certain other bacterial and mycotic infections, as well as with some helminthic infestations, a form of hypersensitivity often appears which may be shown by skin reactions of various types. Of these, the trichophytin test in fungal infections, and the Cassoni test in hydatid disease due to the echinococcus, are those most often employed. The use of various skin reactions as diagnostic procedures in parasitic diseases is discussed by Gay (1935).

Particular attention has long been directed to the development of hypersensitivity during the course of streptococcal infections. The common association of acute infections with hæmolytic streptococci and the clinical manifestations of acute rheumatism and nephritis has naturally suggested to many observers the possibility that these bacteria may occupy an important ætiological

rôle in these diseases. The failure to recover streptococci from active lesions in the heart and kidneys in such cases, which has been the experience of most investigators of these problems, together with the typical interval of ten to twelve days between the onset of the usual pharyngeal angina and the rheumatic or nephritic signs—the period characteristic for the formation and release of antibodies—has led many to regard the cardiac, vascular, articular and renal lesions as allergic in nature. It is supposed that certain connective tissue elements in the heart, joints, kidneys and elsewhere become sensitized by streptococcal antigens that are absorbed through the inflamed mucosa of the throat, and that the subsequent formation and liberation of antibodies into the circulation leads to an allergic inflammation where the two unite. Should this belief be correct, it would be the antigen which would first attach itself and become sessile on the cells, and the reaction would follow the arrival of the specific antibody. The parallel with serum sickness so far as pathogenesis is concerned will be obvious. Further reference to this concept will be made below.

Various Types of Allergic Reactions seen in Hypersensitive Persons

General Principles

The clinical and pathological manifestations of allergic reactions are exceedingly varied, both as regards the rapidity of their appearance after re-exposure to the specific allergen and the particular tissues or group of tissues which form what Doerr has termed the "shock organ." Although no comprehensive explanation for these variations is yet possible, certain broad principles have emerged from the confusion of detail, and it is becoming increasingly evident that it will be mainly along immunological lines that answers to the many outstanding questions will be found. It will be well, therefore, to begin a discussion of the more important "immediate" and "delayed" allergic reactions met with in clinical practice and their morbid histological features, with a brief review of some of the relevant general principles. For the sake of clarity they will be set out in the following propositions :

A. *Allergic reactions only take place in a tissue that has become sensitized through the attachment to its cells of some specific sessile antibody or antigen.* Usually this sensitization is generalized to the whole body, the provocative antigen and its specific antibody

having become widely distributed by the circulation. An intravenous injection of antitoxic horse serum or a primary infection with the tubercle bacillus, for example, both lead to a state of generalized hypersensitivity, as can easily be shown by specific tests in the skin on any part of the body. Although commonly of this wide distribution, under certain circumstances the field of sensitization may be more restricted in area. Sometimes, when exposure to the antigen is limited to some particular site, such as the respiratory mucosa with pollens and dandruffs, or the skin with chemical allergens, specific antibody formation may be largely concentrated in the area primarily stimulated. Walsh and Cannon (1938), for instance, showed that the application of a bacterial vaccine to the nasal mucosa in rabbits led to the predominant formation of specific antibody in the local submucosal tissues. Similarly, Oakley and his colleagues (1949) found that the injection of tetanus and diphtheria toxoids into opposite hind legs of horses was followed by corresponding differences in the amounts of the specific antitoxins that could later be extracted from the skin and lymph-nodes close to the two inoculation sites. It has frequently been observed also that when the skin has been exposed to some sensitizing chemical, hypersensitivity is often, at least at first, limited to that particular area alone (see Sulzberger, 1940). Should occasions for exposure multiply, however, as with an industrial hazard, sensitization tends to become generalized. A second reason for the occasional inequality of distribution of immune bodies is their tendency to accumulate in inflamed structures, irrespective of whether the inflammation is a specific one or not. As is well known, capillaries in inflamed tissues are abnormally permeable to proteins, so that any sensitizing antigen or antibody circulating at the time would tend to gravitate to such areas (see Auer, 1920). It seems likely also that in persistently inflamed tissues the accumulation of macrophages may so augment the local antibody-forming apparatus that local sensitizations of the kind described for the respiratory tract by Walsh and Cannon may be more prone to develop in consequence.

B. *The clinical manifestations of a hypersensitivity reaction depend only indirectly on the chemical nature of the allergen.* It is characteristic of all substances which exhibit "primary toxicity" that their entry into the tissues gives rise to a syndrome of a distinctive kind. The alkaloids, morphine, strychnine and atropine, for example, all possess their own characteristic toxi-

cological properties. No such differentiating features characterize the actions of the allergens, however, which operate not by virtue of any inherent primary toxicity—of which in most instances there is none—but because of the disturbance in cell function and the release of pharmacologically active substances which follow from the combination of antigens and antibodies when either has become incorporated in a cell membrane. In short, all allergens possess the common property of being either full antigens themselves or of possessing the property, as haptenes or pro-antigens, of being able to couple with host tissue proteins and thus acquire the character of full antigens. It is this unifying link between antigens and pro-antigens that connects, for instance, the "serum sickness" caused by diphtheria antitoxin given intravenously on the one hand with that following sulphaguanidine taken by mouth on the other (see Longcope, 1943). From such different origins the chain of processes in each instance converges finally on a common link and thus a common toxicological pattern—an antibody-antigen reaction in which one or other is sessile on vulnerable cells.

C. *The clinical manifestations of allergy are largely determined by the situation in the body of sessile antigen or antibody, and their accessibility to the corresponding specific immune body.* As has already been stated, even localized exposure to an antigen if prolonged gives rise to a state of generalized hypersensitivity. This widespread distribution of the specific immune bodies is clearly shown by the finding that most persons who suffer from pollen asthma, foreign serum reactions or hypersensitivity to foods, such as fish or egg, also possess sufficient sensitivity in the skin for the intracutaneous injection of saline extracts of the offending substances to evoke acute local reactions. Such persons, although generally sensitized, show symptoms which are mainly or exclusively limited to the respiratory or alimentary tract. This is clearly due to the fact that under natural conditions of exposure the specific antigen reaches and expends its force primarily on one or the other of these organ systems. When the sensitizing antigen has been introduced into the blood-stream, as with intravenous injections of antitoxins in the treatment of diphtheria or tetanus, the tissue sensitization becomes particularly diffuse. It is because so many shock organs are affected simultaneously, and so large an amount of histamine is released too rapidly for the tissue histaminases to destroy it sufficiently quickly, that anaphylaxis is so grave an emergency in serum therapy.

How dependent the state of hypersensitivity is upon the presence of sessile immune body is shown very persuasively by a variant of the well-known Prausnitz-Küstner reaction which was introduced by Walzer (1927), and has since been repeated by many others. If a small area of the skin of the forearm of a normal person is passively sensitized by the inoculation of 0·1 ml. of serum from a patient allergic to fish, egg or other food, the ingestion of the offending food by the recipient some hours later is promptly followed by a " triple response " at the inoculated site. The transferred serum contains specific sensitizing antibody, which shortly after its inoculation becomes anchored to the local skin cells, so that when the allergen is ingested and absorbed in traces through the intestinal mucosa, its carriage in the circulation to the forearm results in a diminutive local passive anaphylactic shock at the previously sensitized site.

In most allergic reactions it is the antibody which is sessile on the sensitized cells, and the later advent of the allergen which fires off the response. There is one well-known condition, however, in which this order is reversed. This exception to the general rule is of such great practical and theoretical importance that it requires more than a passing reference. After the inoculation of therapeutic doses of ordinary horse serum antitoxin, no untoward sequel may appear for six to twelve days (see Kojis, 1942). Rather suddenly at the end of this period, large areas of scarlatiniform erythema may appear on the trunk and limbs, especially near the site of the injection, and are rapidly followed by equally large " geographical " patches of itching urticaria. After persisting for an hour or two, these affected patches subside, to be followed by similar eruptions elsewhere, until, region by region, most of the surface of the body has been covered. Sometimes the skin manifestations are accompanied by acutely painful swelling of the joints, with mild fever and albuminuria ; occasionally a temporary paresis affects the arms and persists for some days (see Bennett, 1939). The severity of the attack is closely related to the crudity of the antitoxin ; with modern refined antitoxic globulins, serum sickness is infrequent. It has not, however, been entirely obviated even with the newer fractionated sera, and may still be encountered in patients who have received large doses or who have been given serological treatment on some former occasion.

Early in the days of diphtheria antitoxin therapy, when serum sickness was common in fever hospital practice, von Pirquet and

Schick (1905) carried out a classical study of the several different clinical types of reaction that might follow serum injections, and the explanations they advanced for their pathogenesis have now been generally accepted.[1] In the six to twelve days between the injection and the rash, the serum proteins are believed to become distributed in the tissues in two ways: firstly, to the tissues generally (where they become incorporated in the membranes of cells) and, secondly, to the reticulo-endothelial system (where they act as antigens). From these latter cells, specific antibodies are released into the circulation about a week after the injection of the antitoxin. Escaping from the blood into the tissue fluids, these newly-formed antibodies eventually reach the sessile portion of the antigen which has become anchored to the cells; it is the union of these two immune bodies in the cell membrane which injures the cells and provokes the typical erythematous and urticarial eruption. Formerly, patients with serum sickness used sometimes to suffer one or more relapses before the condition finally subsided, and there are grounds for supposing that these coincide with the successive outpourings of antibodies to the different albumin and globulin constituents of the foreign serum injected (see Davidson, 1919).

Disturbances indistinguishable clinically from serum sickness are not seldom met with after treatment with arsphenamines and sulphonamides (Longcope, 1943) and penicillin (Gordon, 1946). In these complications of therapy, the same underlying immunological mechanism seems to be at work—the drug-homologous protein complex occupying the *rôle* of antigen usually taken by the protein of the foreign serum.

The Varying Rates of Appearance of Allergic Reactions.

A notable and still obscure feature in the clinical manifestations of specific reactions in already sensitized individuals is the great variations in the intervals that elapse between the local injection of the antigen and the appearance of the erythematous responses. In certain circumstances, the reaction develops within a few minutes, reaches its climax in a quarter of an hour, and disappears without trace in two hours. Such reactions are appropriately termed "immediate." In the others, which are described as

[1] Von Pirquet and Schick's short monograph on serum sickness has recently (1950) been translated into English. It is one of the great classics of Immunology, and should be read by anyone interested in the scientific analysis of hypersensitivity states.

"delayed," a latent period of twelve to twenty-four hours may separate the re-exposure to the antigen and the erythema it excites. While much is now known of the mechanisms which underlie the immediate or anaphylactic type of response, great uncertainty still surrounds the pathogenesis of reactions of the delayed type. It seems likely that this will continue until fuller knowledge has been gained of the nature of the pharmacologically-active vasodilator substances that are released in this latter form of allergic inflammation.

Immediate Reactions. The most classical type of immediate response, and the one most studied because of its serious danger to life, is anaphylaxis. Although man is not amongst the more vulnerable species in this respect, many instances of fatal anaphylaxis in human beings have been recorded (see Rutstein *et al.*, 1941 ; Kojis, 1942 ; Ratner, 1943). Most of these deaths took place at the time when *crude* horse serum antitoxin was still widely used in the treatment of diphtheria and pneumonia ; with the introduction of modern, concentrated, enzyme-treated ("*refined*") antitoxic globulins, the risk of serious reactions of this kind has much lessened. But although the danger for ordinary persons is now very slight, there should be no relaxation in the precautions formerly customary for the administration of serum. Especial care is needed with patients who give a history of asthmatic attacks, for in man, as in the guinea-pig, a prominent feature in many cases of fatal anaphylaxis is asphyxia due to severe constriction of the bronchi. The principal measures to be taken to reduce the likelihood of mischance in serum therapy are : the test for any unusual specific hypersensitivity in the patient by undertaking a preliminary intracutaneous test with diluted horse serum ; the avoidance as far as possible of the intravenous route of administration ; the slow infusion of the serum in warmed saline solution ; and lastly, preparations ready for the immediate subcutaneous injection of adrenalin (0·5 c.c. of 1 : 1000 *Injectio adrenalini*, B.P.) should any untoward sign appear during or shortly after the administration (see Parish (1951) for practical precautions required in serum therapy).

In the great majority of instances, anaphylaxis in man is of the direct or active kind, but a few cases have been reported in which it has assumed the form known to immunologists as "reversed passive anaphylaxis." Kellett (1936), Voss (1937–38) and Karalitz and Stempian (1942) have all described clinical shock phenomena in patients who, after having received a therapeutic or

prophylactic injection of diphtheria or tetanus horse serum antitoxin, were given quite by chance a blood transfusion from a person *who had previously become sensitized to horse serum proteins* (almost invariably as the result of some therapeutic or prophylactic injection of serum months or years before). Under these circumstances, the first injection provided an antigen (horse serum proteins), part of which became attached to cells of shock organs, while the second injection—the blood transfusion—of human anti-horse serum supplied the specific antibody which, by union with the now sessile antigen, provoked the shock. Although rarely met with in the past, the increasing use of a combination of serum prophylaxis and blood transfusions, especially after serious road accidents, are likely to render this form of anaphylaxis more common in the future.

Of less gravity than anaphylaxis, but almost as immediate in their onset, are many cases of hay fever and asthma due to sensitization with grass pollen or animal dandruff. The amount of antigen needed to precipitate such an attack is often minute ; the mere approach of the allergic subject to the provocative plant or animal may lead within a few minutes to respiratory discomfort and rhinorrhœa ; and shortly afterwards to an attack of asthma. In these cases there seems no reason to doubt that the shock dose of the antigen is inhaled in the form of finely-divided particles, which, dissolving on the bronchial mucosa, allows the specific protein to be absorbed, and to come into immediate contact with the sensitized structures beneath.

Almost equally prompt in occurrence are some of the allergic manifestations to ingested antigens. Sometimes within a few minutes of the offending food entering the mouth, the buccal mucous membrane may become conspicuously swollen. Usually, however, the clinical signs appear less quickly, and affect chiefly the stomach and intestines. Abdominal discomfort, followed by vomiting, may take place within an hour, and may soon be succeeded by colicky pains and diarrhœa. The effect of an ingested allergen on the gastric and colonic movements of a hypersensitive person has been followed by Hampton (1941), who mixed a radio-opaque material with some of the offending food. Although the spasticity of the colon is much increased by the allergen, it is likely that the mucous membrane also reacts to its presence. Gray and his co-workers (1939–40) studied the effect of the ingestion of food containing an allergen on a small patch of mucous membrane which had been exteriorized near a colostomy opening

and which had been passively sensitized by the injection of a small amount of specific serum obtained from an allergic subject. In this experiment, which was analogous in principle with the Prausnitz-Küstner reaction, the sensitized area showed hyperæmia, swelling and excessive secretion of mucus within a few minutes of the ingestion of the antigen. So prompt was the response that the antigen involved must have been absorbed in a reactive form in the upper part of the alimentary tract and carried to the colon by the blood-stream.

Delayed Reactions. In some important forms of allergic reactions, especially those of the skin, the signs of tissue injury, as indicated by incipient erythema, do not begin to appear for twelve to twenty-four hours, and usually only reach their maxima some forty-eight to seventy-two hours after exposure to the antigen has occurred. Such reactions, known as "delayed" reactions, may assume one of two clinical forms :

i. That typified by the intracutaneous Mantoux tuberculin response, in which the skin becomes erythematous with a rather cyanotic tinge, and
ii. That which commonly follows contact with simple chemical pro-antigens and which is characterized by its eczematous nature.

In spite of the inability of almost all investigators to transfer sensitivity from persons who present delayed reactions by the Prausnitz-Küstner method, there can be no doubt that the sensitization must depend upon some host response of an immunological nature. Only in this way is it possible to account for the simultaneous appearance of tuberculin sensitivity of the skin over the whole surface of the body of a person or animal that has contracted a tuberculous infection. Even in contact dermatitis, the area of cutaneous hypersensitivity, which at first is confined to the immediate vicinity of the primary application of the antigen, soon extends to the skin of the body generally. Yet in persons in whom vigorous delayed reactions develop either through some fortuitous re-exposure to the specific allergen, or are evoked intentionally for diagnostic or experimental purposes, serum antibodies able to bring about a precipitin test *in vitro*, or of passively transmitting local sensitivity *in vivo*, are seldom demonstrable. In these respects, the sera of patients who show delayed "tuberculin-like" or "contact eczematous" types of responses differ from those of patients who present the "immediate" type

reactions of hay fever or urticaria, in which the allergen is typically a food, organic dust or foreign serum protein.

In spite of much work, the immunological factors which determine whether a skin reaction shall be immediate or delayed are still obscure. There are reasons for believing that the character of the response is largely independent of any peculiarity in the antigen itself. Lewis and Seibert (1931), for instance, have shown that the specific protein extractable from tubercle bacilli, and on which the delayed tuberculin test depends, can evoke typical anaphylactic shock in guinea-pigs, and Seibert (1932) later also prepared specific precipitating sera to this protein in rabbits. Similarly, specific precipitating sera can be made against a number of chemicals which are able to excite contact eczema if these are injected intraperitoneally in rabbits instead of applied to the skin (Gell *et al.*, 1946).

It now seems likely that the character of an allergic response may be mainly determined by some peculiarity in the nature of the antibody formed, and that this in turn may depend on the route by which the antigen reaches the tissues. Contact with epidermal cells, as with an industrial chemical allergen, clearly differs materially from a subcutaneous inoculation which would expose primarily the mesenchymal cells of the lower dermis. Whether antibodies formed as a result of differing methods of introducing the antigen differ *qualitatively* or merely *quantitatively* is, however, uncertain, though most immunologists would now favour the former. None the less, the simpler hypothesis of a mere quantitative difference cannot be excluded ; it would explain the association of immediate type reactions with the presence of precipitins in the serum and an ability for passive transfer by the Prausnitz-Küstner technique as reflecting a surplus of specific antibodies in the circulating body fluids. Our methods for detecting such antibodies are still admittedly crude, and were only small amounts to be present in the serum, they might pass unrecognized even with the help of the delicate collodion particle modification of the precipitin test introduced by Cannon and Marshall (1940). On this purely quantitative hypothesis, it is supposed that a weak antigenic stimulus would result in the formation of a correspondingly small concentration of antibody, almost all of which would become attached as sessile antibody to certain cells and thus bring about their sensitization. With stronger or more prolonged antigenic stimulation, antibody would be produced in greater amounts, so that after the sites available

for sessile antibody deposition had been saturated, an excess would still be free in the blood and would protect the sensitized tissues in the event of further exposure to the same antigen. In this way, hypersensitivity would be replaced by protective immunity. It is on these lines, also, that success in the desensitization of hypersensitive individuals by repeated controlled administrations of the specific allergen might be explained.

Most immunologists to-day incline to the view that two or more distinctive kinds of *monospecific* antibody may be produced in response to a single antigen (perhaps because of their elaboration in different portions of the large family of antibody-forming tissues), and that one of these distinctions lies in a difference in the readiness with which they become sessile in the tissues (Burnet and Fenner, 1949). Evidence for heterogeneity of monospecific antibodies comes from several sources, none very convincing alone, but acquiring a certain force when taken collectively. Very briefly, these findings are: the presence of specific antitoxin against diphtheria toxin in two electrophoretically different fractions—the beta and gamma fractions—of the sera of immunized horses (Kekwick and Record, 1941); the greater thermolability of precipitating as compared with sensitizing antibodies (Chase, 1948); the passive transferability of hypersensitivity to immediate reactions by precipitin-containing serum, as by the Prausnitz-Küstner method (Tuft and Ramsdell, 1929), and the failure of comparable conveyance in the case of delayed reactions; the transferability of the delayed, tuberculin-like type of hypersensitivity by injection of *living cells* from an allergic animal (Landsteiner and Chase, 1942; Chase, 1945; Kirscheimer *et al.*, 1947; Lawrence, 1949; Miller and Favour, 1951; Wesslen, 1952; the ability of antibodies capable of passively sensitizing the newborn to immediate type reactions to pass through the placenta, and the failure of those concerned with delayed reactions to do so (Corper and Clark, 1942; Crepea and Cooke, 1948); and the recent recognition of the kinds of antibody known as "agglutinating" and "incomplete" (or "blocking") in the Rh blood groups of man (Race and Sanger, 1950). Some of these points are considered in greater detail by Gell (1945), Chase (1948), and Marrack (1951).

Apart from the still largely conjectural idea of some intrinsic distinction between precipitating and sensitizing antibodies, evidence is accumulating that the clinical form assumed by an allergic response—whether tuberculin-like, eczematous or

urticarial—is largely decided by the manner in which the allergen is re-applied to the sensitized cells. Sulzberger (1940), for instance, has pointed out that simultaneous tuberculin patch and Mantoux intracutaneous tests on the same allergic patient evoke typical delayed eczematous and tuberculin-like reactions respectively. It seems likely that in the patch test the superficial sensitized epidermal cells form the shock organ, while in the latter, the transepidermal inoculation of the antigen causes its main brunt to fall more forcibly on the vascular endothelium of the corium. The skin is a complex organ, and it is possible that differences in the types of cell that form the shock structures may be responsible for differences in the nature and proportions of the pharmacologically-active substances released, and in this way for the distinctive clinical features of the various forms of allergic reactions. This possibility has received some support from the finding that the immediate type response in urticaria can be suppressed or mitigated by anti-histamine drugs such as "Neoantergan" (see Gaddum, 1948 ; Burn, 1948 ; Robson and Keele, 1950), while the delayed reactions of the eczematous and tuberculin-like types follow their course unmodified by their presence.

The Morbid Histology of Allergic Inflammation

The structural changes in tissues undergoing allergic reactions have been much studied in both man and animals. Those in the skin have naturally received most attention because of the ease of biopsy examination, and most of our knowledge of histological changes in allergic inflammation therefore comes from observations on this organ. Laporte (1934) made a detailed study in guinea-pig's skin of both immediate reactions to foreign protein and delayed responses to tuberculin, and his findings, which agree well with those made on man, may be summarized as follows :

In immediate reactions, which reach their climax within a few hours, the small blood-vessels are the tissue elements most affected, for within a few minutes of the provocative inoculation they show dilatation and signs of an escaping exudate. If the reaction is severe, the walls of both arterioles and capillaries are disrupted, and small hæmorrhages appear in the corium. Later the small vessels in the deeper layers of the corium become thrombosed, and the resulting ischæmia adds materially to the severity of the tissue injury. Throughout the earlier phases, both

neutrophil and eosinophil leucocytes accumulate in the swollen corium. The course of these reactions has also been followed in the living tissues of rabbits by Abell and Schenck (1938), who made use of the translucent " ear-window " technique devised by Clark. When horse serum was injected into such specifically sensitized animals, it was found that the arteries were the most reactive elements, for they showed prompt constriction before any other signs were seen. Later an exudate, with many neutrophils, accumulated in the extravascular tissue spaces in the window area.

If the reaction of this immediate type is mild, the vascular injury seldom passes beyond the stage of erythema, increased vascular permeability and local accumulations of neutrophils and eosinophils—the phenomena typical of urticaria in the skin—but when the stimulus is more severe, the local vascular stasis due to arteriolar constriction, thrombosis and loss of fluid from capillaries may lead to areas of necrosis in the dermis. This graver form of allergic inflammation was first described by Arthus in 1903, who observed that multiple closely-spaced successive injections of horse serum into rabbits led to progressively severe responses. The allergic necrosis produced in this way has long been known eponymously as the " Arthus Phenomenon " ; the details of its pathogenesis as described by its discoverer, as well as of its numerous modifications at the hands of later investigators, have been fully reviewed by Nordmann (1931). Though the Arthus Phenomenon is readily evoked in rabbits, it is rarely seen in man, being only exceptionally met with in patients who have received multiple injections of antitoxin (Kojis, 1942 ; Ratner, 1943 ; Kelly, 1946) ; of non-synthetic hormones, notably liver extract and insulin (Harten *et al.*, 1940) ; and of rabbit's brain and spinal cord in rabies prophylaxis (Lepine and Cruveilhier, 1935).

In a histological study on delayed reactions due to tuberculin, which usually reach their maximum after thirty hours, Laporte saw less evidence of injury to blood-vessels than in those of an immediate kind, and more signs of damage to the overlying epithelium, which, with large inocula of tuberculin, often underwent local necrosis. The nature of the leucocytic infiltration, moreover, differed, for although the early immigration was largely neutrophil, macrophages soon greatly outnumbered them ; the former cells only persisted in large numbers if much epidermal necrosis had taken place. Further details of the histology of

tuberculin reactions have been given by Tytler (1930) and Rich (1944).

In vitro experiments with leucocytes and the migrant cells in tissue cultures of the spleen have demonstrated the enhanced vulnerability of cells of tuberculous animals to the specific tuberculous proteins and notably to P.P.D. Studies on these functional lines were begun by Holst (1922), who contrasted the survival times and phagocytic powers of neutrophils from tuberculous and control guinea-pigs in the presence and absence of tuberculin. Later his observations were amplified by Rich and Lewis (1932), who found that the addition of tuberculin to tissue cultures made from tuberculous and normal animals, killed the cells of the former at concentrations which were harmless for those of the latter. Subsequent investigators have not only confirmed these observations, but they have added to them by finding that this raised sensitivity of tuberculous animals' cells *in vitro* has no counterpart in those forms of protein hypersensitivity that are characterized by skin responses of an immediate kind. They have thus provided further grounds for the belief that reactions of the delayed type are associated with an especial tendency for the specific sensitizing antibody to become intimately attached in sessile form to vulnerable cells. Of interest, too, has been the confirmation (Corper *et al.*, 1945) of Holst's observation that the ability of neutrophils from tuberculous animals to phagocyte staphylococci is much impaired in the presence of tuberculin. Tuberculoprotein, acting as a toxallergen, can thus depress the functional activity of sensitized cells. (For recent literature on *in vitro* studies of the reactions of sensitized tissues to tuberculin, see Heilman *et al.*, 1944 ; Miller and Favour, 1951.)

A large mass of confused and often ambiguous information has accumulated upon the effects of hormones on immunological processes. Most of them have centred on possible stimulation of antibody production by various internal secretions ; to a lesser extent the phenomena of anaphylaxis and allergy have come under examination. The recent increase of interest in the potent effects of the adrenocortical steroid hormones on connective tissues has naturally resulted in numerous investigations into the possibility of controlling allergic inflammatory reactions through their use. That the injection of adrenal cortical extracts can exercise some protecting influence in anaphylaxis was first shown by Wolfram and Zwemer (1935) in a series of experiments in which a prior injection of cortin was found to lessen the mortality

of sensitized guinea-pigs into which a shock dose of egg albumin was inoculated. Within the past few years, a number of publications has appeared on the ameliorative effects of the adrenocorticotrophic hormone (A.C.T.H.) and cortisone on such allergic reactions as the Arthus phenomenon (Germuth *et al.*, 1950, 1951 ; Berthrong *et al.*, 1950) and the tuberculin reaction (Stoerk, 1950 ; Long and Miles, 1950). Some of these results—though not all—seem to indicate that antibody production in animals that receive A.C.T.H. is in some degree impaired, so that the allergic reactions are mitigated in consequence (see Fischel, 1950 ; Malkiel and Hargis, 1952). Others suggest that the inhibitory effects of these hormones on inflammatory responses generally may be an important feature of their biological behaviour (Gell and Hinde, 1951 ; Humphrey, 1951 ; Michael and Whorton, 1951). The complexity of the actions of these hormones, however, makes it difficult to evaluate such work at present, or to relate it to the main body of immunological knowledge.

Evidence that small arteries are sometimes seriously injured in the shocked tissues of hypersensitized animals has frequently been recorded since Longcope and Boughton's histological studies thirty years ago. It was only in 1937, however, that Clark and Kaplan described comparable vascular lesions in human beings who had died within a few days of an attack of serum sickness. Since then a number of case reports have appeared in which arterial injuries resembling those found in polyarteritis nodosa (see below) have been described in patients who shortly before death had shown some hypersensitivity reaction to foreign sera, sulphonamides or even iodides (Rich, 1942 ; 1947). There seems little reason to doubt that these lesions are directly dependent on prior sensitization of the arteries, for it is well known that smooth muscle often figures conspicuously as a shock structure in anaphylactic reactions. There is ample evidence, too, from animal experiments that a non-fatal anaphylactic shock may be followed by focal arterial damage (Boughton, 1917 ; Klinge, 1933 ; Rich and Gregory, 1943 ; More and McLean, 1949 ; Sheikh, 1951), and that of the various serum proteins, the albumen seems to be the fraction most clearly concerned in their pathogenesis (Hawn and Janeway, 1947). The significance of these studies in throwing light on the pathogenesis of the arterial lesions of acute rheumatism, polyarteritis nodosa and Bright's disease will be considered in the next few paragraphs.

Hypersensitivity as an Ætiological Factor in Certain Diseases of Man

In several important diseases of obscure pathogenesis, of which rheumatic fever, polyarteritis nodosa and acute nephritis are the most notable instances, the microscopical lesions show certain points of kinship, both with one another and with those found in the more severe allergic reactions in man and animals. Such similarities have naturally suggested that hypersensitivity to some as yet undetermined antigen may occupy a significant place in the ætiology of the whole group—that they may all be united by the common denominator of subacute allergic inflammation. For the present, however, this supposition, which is based on morphological analogies between the characteristic lesions (notably those in which the small arteries show focal necroses and connective tissues undergo localized fibrinoid degeneration of their collagen fibres), can be regarded as no more than a broad working hypothesis to guide further work. In the following paragraphs, some of the evidence which has led to the development of this belief will be briefly reviewed.

Rheumatic Fever. The clinical and epidemiological features of acute rheumatic fever point strongly to an infective origin for the disease, yet the many attempts made by conventional bacteriological methods to recover some specific micro-organism from the heart, joints or other affected tissues have thus far proved inconclusive (see Jones, 1939). Because of the failure of this initial attack upon the problem, ideas on the pathogenesis of these seemingly sterile lesions have been directed into other lines of enquiry (see Waksman, 1949). The first of these hypotheses is that the disease is caused by a virus. Although it is now known that cardiac lesions not unlike those of acute rheumatism can be caused in animals by transmissible viruses (Pearce, 1950), the evidence for a virus origin for rheumatic fever in man is still slight and needs no more than mention here. The second possibility, which has long had a distinguished following amongst immunologists, is that the cardiac and articular manifestations of acute rheumatic fever result from some specific local sensitization of these structures. The argument in favour of this general theory of hypersensitivity, in which no particular antigen is specified—because more than one may well bring about the same result—is based mainly on various points of resemblance between the clinical and pathological features of rheumatic fever

and those of serum sickness (see Klinge, 1933 ; More *et al.*, 1949), and on experiments on sensitized animals (Rich and Gregory, 1943, 1947 ; McKeown, 1947 ; Crawford and Nassim, 1951). Some immunologists, however, would go further and maintain that the specific toxallergen concerned is some soluble product of one or more members of the group of streptococci. Murphy and Swift (1950) and Kirschner and Howie (1952), for instance, have described lesions in rabbits subjected to repeated infections with hæmolytic streptococci which resemble those seen in the hearts of patients dying with acute rheumatic fever.

It has long been recognized that streptococcal acute infections, especially those in the throat, are sometimes succeeded by attacks of rheumatic fever (Glover, 1930). Following up such observations, Coburn and his colleagues (Coburn, 1931) have correlated both epidemiologically and bacteriologically the incidence of rheumatic fever and hæmolytic streptococcal infections. They confirmed that the two conditions have the same seasonal and social class distribution in the eastern United States, and showed that the incidence of attacks of both declined *pari passu* in a small group of rheumatic New York schoolchildren when they were transferred to the warmer climate of Porto Rico in the Caribbean. During the recent war, Coburn and Young (1949) found that the routine administration of sulphonamides for some months to a large number of United States naval personnel greatly lessened (until drug-resistant strains appeared and spread widely) the incidences of both streptococcal throat infections and of rheumatic fever. Kuttner and Reyersbach (1943), also successfully reduced the frequency of relapses in a group of rheumatic schoolchildren in the same way.

Since there is no satisfactory evidence that streptococci are recoverable from any of the lesions in rheumatic fever, even in its most acute stage, the assumption has been made that the injury is brought about by the entry into the circulating blood of some soluble antigenic material formed by the bacteria growing in the throat. A well-recognized clinical feature of the disease, which fits with the hypothesis that this substance acts as a toxallergen and not as a primary toxin, is the characteristic occurrence of the rheumatic manifestations about ten days after the onset of the tonsillitis—an interval that might well be occupied by the formation of specific antibodies. On this general supposition, it is suggested that at sites of stress, such as the heart valves and synovial membranes of joints, the streptococcal

products become localized and thus determine the sites of major injury when the specific antibody emerges later. The parallelism in principle between this hypothesis for the pathogenesis of rheumatic fever and the now generally accepted view of von Pirquet and Schick (see above) on the mechanism of serum sickness is obvious. Its validity, however, is clearly contingent upon the incrimination of the streptococcus as the source of the antigen.

In recent years there has developed an increasing disinclination to regard streptococcal infections as so uniquely connected with acute rheumatic fever as was formerly believed. Several investigators have now shown that a variety of infections, amongst them dysentery, sand-fly fever and malaria (Copeman, 1944), or even trauma (Glazebrook and Thomson, 1941) can provoke an exacerbation of rheumatic fever, so that it now appears that the *rôle* of streptococcal infections may be less specific than was formerly believed, and that it is the commonness of occurrence of such throat infections in temperate climates that have hitherto made this association seem so conspicuous to European and American workers. Moreover, the acceptance of the streptococcus as the sole initiating agent is made more difficult by the lack of any unanimity amongst the many investigators of tonsillitis and rheumatic fever as to the variant form of this bacterium that is concerned.

To meet these and other difficulties, various suggestions have been made that are still within the ambit of the hypersensitivity hypothesis It is now known that substances having the same antigenic properties, and possibly identical in chemical constitution, are very widely distributed in nature (Buchbinder, 1935; Topley and Wilson, 1946 b); noteworthy, and possibly relevant, amongst the many instances of this overlapping specificity now known is that of a common immunological component in the pneumococcus Type XIV and the human Group A red corpuscle. It is by no means impossible that certain tissue proteins, or more probably tissue muco-polysaccharides, such as hyaluronic acid, may present common antigenic characters with constituents of certain bacteria, so that should antibodies be formed against the latter they might at the same time react with the former to the detriment of the host. A second hypothesis that has also invoked a hypersensitization mechanism has recently attracted attention (see Kerr, 1948). It is supposed that for some reason as yet unknown, the patient's own proteins undergo

some change, perhaps comparable with that known to occur when pro-antigens combine with host proteins to excite dermatitis, and as a result become antigenic for their possessor. On this supposition also, the resulting antigen-antibody union would cause focal injuries resembling those of natural and experimental serum sickness.

It will be evident from the above discussion that the inclusion of rheumatic fever amongst the hypersensitivity diseases is still debatable. The whole subject remains very obscure, and it may well be, as Freeman supposed, that the idea of allergy may not be " a gleam of sunshine breaking through, but an extra wisp of fog." Nevertheless, nebulous though it is, the hypersensitivity hypothesis for rheumatic fever is the only one which has yet offered any coherent explanation for the pathogenesis of this very baffling disease.

Polyarteritis Nodosa. This disease, formerly known as periarteritis nodosa, is now more commonly diagnosed during life. Grant (1940) has drawn attention to the great diversity of clinical syndromes under which it may declare itself. Though it has long been known to be associated with such manifestations of hypersensitivity as urticaria and asthma (see Wilson and Alexander, 1945), it is only in recent years that the evidence needed to give serious support to an allergic hypothesis for its pathogenesis has been obtained. Much of this evidence comes from the apparent increase in its incidence that has followed the introduction of new therapeutic methods that are liable to give rise to hypersensitivity manifestations. In a review of the autopsy data of the Johns Hopkins Hospital, Rich (1946–47) has stated that in the twenty years prior to the discovery of the sulphonamides in 1936 there were 6 deaths from this disease in 10,016 post-mortems (0·06 per cent.), but that in the subsequent ten years there have been 38 in 5,207 post-mortems (0·73 per cent.), a twelvefold increase in mortality.

The morphological changes in polyarteritis nodosa are generally confined to the medium and small-sized muscular arteries. They are characterized by small areas of acute inflammation, necrosis of the cellular elements, fibrinoid changes in the collagen, and leucocytic infiltration (for a discussion on the nature of fibrinoid changes, see Glynn and Loewi, 1952). Eosinophil leucocytes usually predominate. Though such discrete lesions may appear in any organ, they tend to be most numerous in the kidney and heart, but owing to their small focal nature they are easily over-

looked if only single sections of organs are examined. Larger arteries are less often involved, though when they are affected the injury to the vessel wall may lead to small sacculations, and these gradually filling with organizing thrombus may in the course of months be converted into nodules of fibrous tissue. Such nodules are occasionally seen to form small grape-like clusters on the surface of the heart. Rarely, in such unsupported vessels as the coronary arteries or the cœliac axis, a necrotic aneurysmal segment may rupture and a fatal hæmorrhage ensue.

It has been pointed out above that in a study of the histological changes in animals that had survived severe anaphylactic reactions, Boughton had found multiple lesions in small arteries that resembled those of polyarteritis nodosa. In recent years these observations have been confirmed and extended (see Roberts *et al.*, 1949), so that no doubt remains that a type of vascular lesion resembling that of the human disease can be produced in animals that have been made sensitive to some antigen. Whether the human disease also has the same pathogenesis is still not wholly clear, but its likelihood has been increased by the observations firstly of Eason and Carpenter (1937), and Clark and Kaplan (1937), and later of Rich (1942, 1947), all of whom have described the occurrence of vascular lesions apparently identical with those of polyarteritis nodosa in patients who have become hypersensitive to foreign sera or to various drugs. With the combined observational and experimental work of Rich and his colleagues, the chain of evidence in favour of an allergic pathogenesis for this disease seems to be approaching completion.

Acute Glomerulonephritis. The very typical occurrence of an interval of about ten days between the onset of scarlet fever and the subsequent attack of acute glomerulonephritis suggested many years ago to Schick the possibility that the renal injury might in some way be brought about by the release of specific antibodies into the circulation. In advancing this idea, he was influenced by the fact that serum sickness, which von Pirquet and he were studying at that time, had much the same latent interval. Since then this possibility has never been lost sight of, but in spite of many attempts to reproduce the disease experimentally in sensitized animals, no one has ever succeeded in producing lesions comparable with those of human acute nephritis by any means that might conceivably be operative in the convalescent stage of scarlatina in man.

An experimental method that has proved successful in the

hands of many investigators for producing a sequence of changes in animals' kidneys that follow very closely those found in human nephritis has developed from the use of specific cytotoxic sera (for literature, see Seegal and Loeb, 1946). When rat or rabbit kidney is finely macerated and injected into a foreign species, a potent nephrotoxic serum can be produced, which on inoculation into the original species leads to serious renal changes. The lesions brought about in this way are initially glomerular in situation, but they progress through a series of changes that morphologically resemble those typical of human nephritis as it advances from the acute to the chronic form.

Although it is clearly impossible to account for human glomerulonephritis as a direct response to the action of any foreign cytotoxic serum, many experimental pathologists have been reluctant to abandon the belief that some immunological mechanism is at work in the pathogenesis of the disease. Schick's hypothesis of forty years ago still remains credible and attractive. Any such mechanism, however, could only take the form of some auto-immunization of the individual against one or more elements of his own kidneys. Speculations of this kind are not modern—they go back to Ehrlich's pioneer work on hæmolysis at the beginning of the present century—but hitherto, in spite of many attempts, no authentic auto-antibody to an unchanged host protein has yet been found, with the exception of that of the lens protein of the eye. Whether the modification of this auto-immunization hypothesis engendered by Landsteiner's work on the antigenicity of a host's own proteins when these have been altered by the addition of some pro-antigen will prove more rewarding will remain for future study. Already attempts have been made to confer such changes on renal proteins by their exposure to contact with streptococci, but these have so far met with no success (Humphrey, 1948). Although the whole problem is of exceptional difficulty, Landsteiner's pioneer studies on auto-immunization to *partly-modified* host proteins seems to offer one of the most promising lines of enquiry along which to seek for its solution.

Although the concept of hypersensitization as a stage in the pathogenesis of certain disease processes is still in many respects very nebulous, at least three important principles have already emerged ; firstly, that many therapeutic procedures now in common use can lead to the sensitization of an important fraction of the individuals undergoing treatment ; secondly, that a shock

response that results from hypersensitivity can entail serious and widespread injuries in very vital structures ; and thirdly, that whenever the ætiology of any acute inflammatory reaction is in question, careful consideration must in future be given to the possibility that it may have resulted from the operation of some toxallergen on a sensitized tissue rather than from the direct action of a primarily toxic agent. The next decade will probably see a considerable clarification of ideas that are now confused, but this will only be accomplished through careful work in which the features of naturally-occurring allergic diseases in man and experimentally-induced sensitization lesions in animals are correlated by investigators who ground themselves properly in modern experimental pathology and immunology as well as in clinical medicine.

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CHAPTER III

THE RETICULO-ENDOTHELIAL SYSTEM

THE expressions " *Reticulo-Endothelial System* " and " *Reticulo-Endothelial Metabolic Apparatus* "—of which only the former has survived, although the latter has much to recommend it—were coined by Aschoff and Landau in 1913 as alternative generic terms for a number of types of widely distributed mesenchymal cells, all of which are characterized by the readiness with which they phagocyte small particles and coarse colloids. Aschoff's contribution, which is clearly summarized in his " Lectures on Pathology," was mainly the unification of many observations made by earlier histologists on the occurrence in many different organs and tissues of cells that possess this distinctive ability. It was this functional attribute of phagocytosis, displayed as he stated with " intensity and frequency," that Aschoff used as the primary basis for his classification. By doing so he simplified a terminology that was becoming cumbrous with terms that were nearly if not quite synonymous. Most of the names given by earlier histologists are now seldom used, and the terms " *reticulo-endothelial cells* " (derived from their predominantly endothelial appearance and believed ability to form reticulin), " *macrophages*," " *histiocytes*," together with " *monocytes* " in the blood and " *Kupffer cells* " in the liver, alone remain in common use. In some respects Aschoff's synthesis may in future prove to have been an over-simplification, for the employment of a few names for what may turn out to be a heterogeneous group of cell types (even though all are distinguished by the common property of phagocytosis) may obscure important secondary differences amongst the members of the system. For the present, however, the classification is valid and convenient.

The functions that are already known to be carried out by this widespread system of cells are numerous and important ; after outline here, they will be more fully considered below Firstly, as Metchnikoff (1905) constantly emphasized, the macrophages, both those lining vascular channels and those present in the fixed connective tissues, form one of the most effective defences of the body against infection—a protection which is now known

to operate in infections with micro-organisms of many different types. Closely associated with this destruction of invading microbes is the important *rôle* of the reticulo-endothelial system in the formation of those modified plasma globulins known as antibodies which react specifically with such living agents and some of their metabolic products. Secondly, those parts of the system that line the blood sinuses of certain viscera normally undertake the katabolism of the hæmoglobin of senile red corpuscles and its conversion into bile pigments and hæmosiderin. For this reason, the system occupies an important place in the metabolism and conservation of iron. Thirdly, the reticulo-endothelial cells seem to participate in a way not yet clearly understood in the synthesis and storage of certain lipoids, for in some disorders of the system its cells become laden with excessive amounts of these substances.

The Identification of Reticulo-Endothelial Cells in Tissues

Since the definitive attribute of the reticulo-endothelial system is the functional one of ingestion of particles and colloids, the primary identification of its constituent cells can only be safely undertaken with the aid of *intra vitam* methods of staining. The materials used for this purpose must therefore be devoid of toxicity as well as strongly and distinctively coloured. Of the many particulate suspensions tried, those prepared from india ink, carmine or finely-divided graphite have proved the most satisfactory, and much of the mapping of the distribution of the component cells of the system has been carried out with their aid. Since the individual particles in such suspensions are too large to escape readily from capillaries, however, such pigmented materials are for practical purposes chiefly suited to the identification of those reticulo-endothelial cells that line blood and lymph sinuses, or that occur in aggregations close to the serosal membranes of the large body cavities. While for cells in these situations such coloured suspensions afford a very effective method of display, those macrophages that lie in the depths of the tissues can only be readily identified by the use of stains that are capable of passing through the endothelium of blood capillaries into the extravascular tissue spaces.

The second method referred to above depends upon the injection into living animals of certain diffusible, non-toxic, stains, of which trypan blue, brilliant vital red and Evans blue are notable

examples. Why only a small proportion of chemically closely similar dyes should be effective for this purpose is not yet fully understood. Recent work has shown, however, that some, and perhaps all, of those dyes that are capable of vitally staining macrophages have the property of becoming rapidly and firmly bound to the albumin of the plasma and tissue fluids. Rawson (1943) has shown by both electrophoretic and ultracentrifugal methods, that trypan blue and Evans blue become promptly attached to this constituent of the plasma, though no comparable fixation occurs with any of the plasma globulins. It is probably because they form such firm complexes with the colloidal plasma albumin, and therefore only escape slowly through the walls of the smaller blood-vessels (Abell, 1940), that these dyes—particularly Evans blue—have proved to be particularly valuable for the rapid determination of blood volume in man. The attachment of the dye to the protein, however, appears to modify the albumin in some way, for if animals on which such blood volume studies have been made are allowed to survive for several days, coarse protein-dye aggregates become visible in the macrophages lining the blood sinuses of the liver, spleen and some other organs. Some of these acidic benzidene dyes, such as trypan blue, are less rapidly attached to the plasma albumin than Evans blue (Gregersen and Rawson, 1943), so that part of the injected stain escapes into the tissue fluids. There it couples with such albumin as these fluids contain and becomes ingested by the extravascular macrophages, with the result that these components of the reticulo-endothelial system as well as those lining blood-vessels become clearly recognizable histologically. It is probably because it combines these two properties of diffusibility into the tissue fluids and a capacity for firm attachment to albumin that intravenously injected trypan blue has been found to be such a valuable dye for the identification of both vascular and extra-vascular macrophages.

Before ending this brief outline of the principles that underlie the *intra vitam* staining of the reticulo-endothelial system by certain acidic dyes, attention may be drawn to the remarkable ability possessed by certain of the plasma proteins, and especially by albumin, for the formation of firm complexes with a number of small molecular weight substances in addition to these dyes and the bearing that this has on some problems in pathology (see Edsall, 1947). Bennhold (1932), who made pioneer studies on the differing diffusibility of various substances into gels in the

presence and absence of serum, found that not only were the movements of certain dyes retarded in consequence of their fixation to the large protein molecules, but that bilirubin and cholesterol were similarly bound. Since then the list of such complexes has been extended considerably. For example, Hamilton Fairley (1941) found that free hæmatin, which appears in the plasma in profound hæmolytic crises such as occur in malaria, becomes rapidly coupled to albumin to form the abnormal blood pigment methæmalbumin, but fails to form similar combinations with any of the normal serum globulins. Recently, Davis (1943), in an interesting review of the factors that affect the distribution of drugs in the body fluids, has described the close binding of the important sulpha-drugs to the plasma albumin. It may be noted here, too, that this fixation may not only retard the escape of these therapeutic agents into the tissue fluids, but that it may also account for the occasional immunological sensitizations that follow their use.

The Distribution of Reticulo-Endothelial Cells in the Body

Through the use of the staining methods just described, and by their correlation with findings upon human beings suffering from various diseases such as malaria, in which distinctive pigments are formed in large amounts, the distribution of the reticulo-endothelial cells in normal organs and tissues has been fully mapped. Beautiful coloured illustrations showing the appearances and histological arrangement of these cells have been published by Cappell (1929). As their name implies, most of the constituent cells are endothelial in form, and are to be found lining blood sinuses in various organs, but more particularly in the liver, spleen and bone marrow, as well as the lymphatic sinuses in lymph nodes. Other cells of the system, sometimes referred to as "resting wandering cells," lie either singly or in microscopical aggregations in the interstices of the connective tissues in many parts of the body. Though these latter cells, as seen in normal tissues, appear to belong to the category of "fixed cells," they are none the less able to migrate actively under chemotropic influences should the structures they reside in be disturbed by injury or infection.

Of particular interest because of their importance in infections of the large serous sacs, are the aggregations of macrophages that occur at certain sites in the subserosal tissues of the peri-

toneum and pleura. In the peritoneum, collections of these cells in varying stages of maturity are present in the omentum and mesentery, and since to the naked eye these masses often appear as fine white streaks close to small lymph trunks, they have long been known as "*taches laiteuse*." Should India ink or finely-divided carmine be injected into the peritoneal cavity these clusters of macrophages become deeply coloured by ingested particles. If infection of the sac takes place, these aggregations, especially those in the ordinarily motile omentum, form important reservoirs from which large numbers of defensive macrophages migrate into the exudate and engulf invading bacteria. In the pleura, similar collections of phagocytic cells are present in the mediastinal folds, and Cooray (1949) has recently studied experimentally in rabbits the reactions of these foci to particulate matter introduced both into the cavity itself and into the nearby mediastinal tissues. In either case, the particles are carried largely in consequence of the tissue fluid drifts brought about by respiratory movements, to these subserosal aggregations of macrophages, whence their further dissemination is restricted by phagocytosis.

Although the use of coloured suspensions and dyes provides a very adequate picture of the histological arrangement of reticulo-endothelial cells in organs and tissues, such methods can only provide a very rough evaluation of their proportionate distribution between the major viscera. Such quantitative data are needed, especially for the macrophage cells lining the blood sinuses, if any estimate of the extent of participation of the various major organs in the phagocytic and metabolic processes of the system is to be attempted. Some early observations on these lines were made by Drinker and Shaw (1921), who injected colloidal manganese intravenously in cats, and later estimated by quantitative chemical methods the amounts of this metal that could be recovered from the major viscera. Similar experimental studies upon the proportionate distribution of foreign proteins labelled with the tracer element arsenic (Haurowitz and Breinl, 1932), of "Thorotrast" (Gaunt and Payling Wright, 1940), or radioactive chromium phosphate suspensions (Jones, Wrobel and Lyons, 1944), have given much the same results. In rabbits, the species most used for such experiments, about half of such foreign material is removed by the reticulo-endothelial cells of the liver, one-quarter by those of the bone marrow and one-eighth by those of the spleen. It is clear from these findings that the great bulk

of the macrophage system that protects the body in bacteriæmias resides in these three organs. Such studies can, of course, give no indication of the relative numbers of extravascular macrophages or " resting wandering cells " in the various viscera and tissues.

In some organs, notably the lungs and the central nervous system, the relation of the reticulo-endothelial cells to certain other distinctive component cells has given rise to much controversy. In the lungs, the common finding of dust particles, hæmosiderin and bacteria in large cells in the interior of the alveoli has led to the belief that these cells play an important part in the disposal of foreign particles in the lungs. The elucidation of the origin—or possibly the origins—of these cells has proved difficult, however, and several different sources have at various times been suggested (see Robertson, 1941). There now seems general. agreement that the earlier beliefs that they are modified vascular endothelial cells or alveolar lining epithelial cells were erroneous. One or more of the following three derivations are now considered to be more probable : the distinctive " *septum cells* " that lie in the pulmonary interstitial structures, which Clements (1940) believes to be the precursor of the " *dust cells* " and " *heart-failure cells* " in the alveoli ; resident reticulo-endothelial cells that lie in the local connective tissues ; and blood-borne deciduous macrophages which have been carried to the lungs after they have been shed from their sites of origin in the liver and other reservoirs of the system. That showers of macrophages appear in the systemic venous blood-stream was shown long ago by Simpson (1922), who found, by differential white cell counts of the blood in the right and left sides of the heart, that large numbers of circulating macrophages are filtered from the blood during its passage through the lungs. The belief that the phagocytes of the lungs are constantly replenished from extra-pulmonary sources of macrophages is further supported by some observations of Rous and Beard (1934). By passing the perfusion fluid from a liver, whose reticulo-endothelial cells had previously ingested fine particles of metallic iron, across the poles of a powerful magnet, they were able to obtain a separation of the liberated macrophages, whose behaviour on subsequent cultivation *in vitro* they found to bear a close resemblance to that of many of the pulmonary phagocytes. Thus it seems likely that, irrespective of any contribution made by the " septum cells " or resident reticulo-endothelial cells to the protection of the lungs against bacteria and dust, the macrophage defences of

these organs are being constantly reinforced by the transport of such cells from the main portions of the system elsewhere in the body.

It has long been recognized that certain neuroglial cells possess the property of ingesting India ink and trypan blue when these have been inoculated into the brain substance. These "*microglial cells*," to use the term given to them by Hortega, are now regarded as the central nervous system representatives of the reticulo-endothelial system. In injuries to the brain and spinal cord, microglial cells respond promptly by ingesting cell and interstitial debris. Moreover, the cells that accumulate in large numbers round brain infarcts, and which have long been known as "*compound granular corpuscles*" or "*gitter-zellen*" are no other than lipoid-glutted microglia. In poliomyelitis, in which many of the nerve cells of the anterior horns of the cord may be destroyed by the virus, microglial cells may be found in large numbers acting as "*neuronophages*" by ingesting the remains of the necrosed neurones. Though their kinship with the reticulo-endothelial system has now been established, the embryological derivation of the microglia has given rise to much discussion. It now seems likely, however, that the view originally advanced by Hortega, and since supported by Dougherty (1944), that microglial cells are derived from undifferentiated mesenchyme in the pia arachnoid and migrate into the neuroglia during fœtal and early post-fœtal life is correct.

The Functions of the Reticulo-Endothelial System

Defence against Infection. In the past, the chief interest in the functions of the reticulo-endothelial system has centred round its contributions to the defences of the body against infection, and this would still be generally accepted as the most important of its activities. At first, when this belief still met with widespread criticism, interest was mainly confined to accumulating the morphological evidence in its favour that was provided by the many instances in which the cells of the system could be observed in the act of ingesting and destroying invading bacteria. In this line of investigation, Metchnikoff occupied a dominating place, and it was largely through his untiring efforts in the field of comparative pathology that the significance of the process of phagocytic digestion of micro-organisms in the higher animals came to be appreciated. Later, however, a second and no less important defensive function of the system became apparent—

the part taken by its cells in the elaboration and release of those specialized plasma globulins known collectively as specific antibodies. With the progressive developments of immunology, the close dependence of defence against invading microbial parasites upon both these cells became fully realized, so that the earlier sharp divisions between the "Cellular School" and the "Humoral School" are now recognized to be wholly artificial. None the less, although the participation of the reticulo-endothelial system in defence against infection is essentially a unitary response, it is a complex one which displays at least two distinct and successive phases, namely, that of phagocytosis and that of antibody formation. In an analytical discussion of the defensive *rôle* of the reticulo-endothelial system, these two aspects are best treated separately.

(*a*) *Phagocytosis of Micro-organisms.* The results of many of the early studies on the ingestion of bacteria by tissue phagocytes were brought together by Metchnikoff in his "Immunity in Infective Diseases," a book which will long remain one of the classics of medicine. About the same time, Wyssokowitsch (1886) described his pioneer studies on the mechanisms by which bacteria are removed from the circulating blood, and thus demonstrated the effectiveness of the reticulo-endothelial system in suppressing bacteriæmias and septicæmias. During the present century, the same ground has been traversed by many investigators, and our knowledge has been enlarged both by the experimental study of an increasingly broad range of parasites and by the payment of greater attention to the comparable tissue responses found in naturally-occurring infections in man. As might be expected, bacteria have figured most prominently in such investigations, not only because as a class they form the largest and most important of microbial enemies, but also because of the comparative ease with which their fate in the tissues can be followed histologically. With the identification of a number of protozoa and fungi as pathogenic agents, however, instances of the phagocytosis of these agents too by the reticulo-endothelial system have been increasingly recognized and investigated. Since much of the older work on the reactions of the macrophages in bacterial infections is now to be found in the text-books of Pathology and Bacteriology, a few instances will be outlined here of protection against these other types of parasite. Such examples will illustrate the adaptability of the reticulo-endothelial system in the defence of the tissues against a wide variety of microbial pathogens.

In malaria, not only the free plasmodia, but also many of

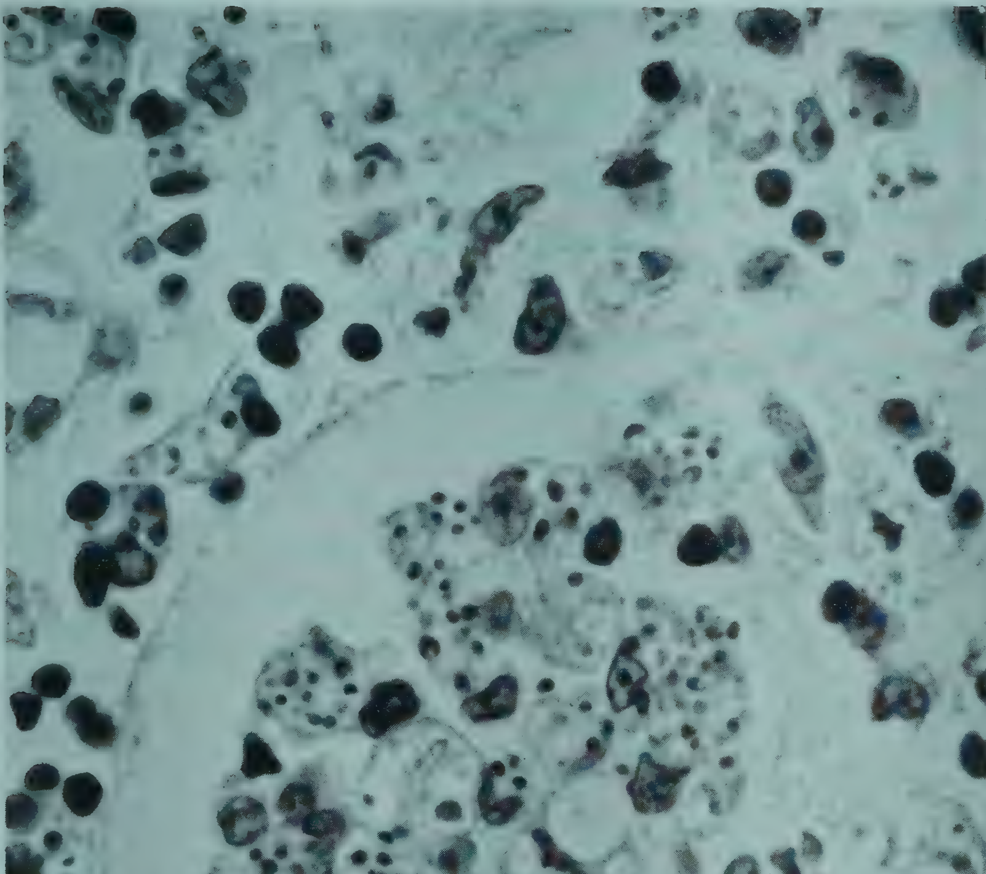

FIG. 3/1. Spleen in Histoplasmosis. From a fatal case with widespread involvement of the reticulo-endothelial organs. A venous sinusoid containing a clump of greatly enlarged macrophages, all of which contain spherical fungus bodies growing freely in the cytoplasm of the phagocytic cells. × 1000. (*Photomicrograph kindly supplied by Dr. George J. Cunningham.*)

[*To face page 66.*

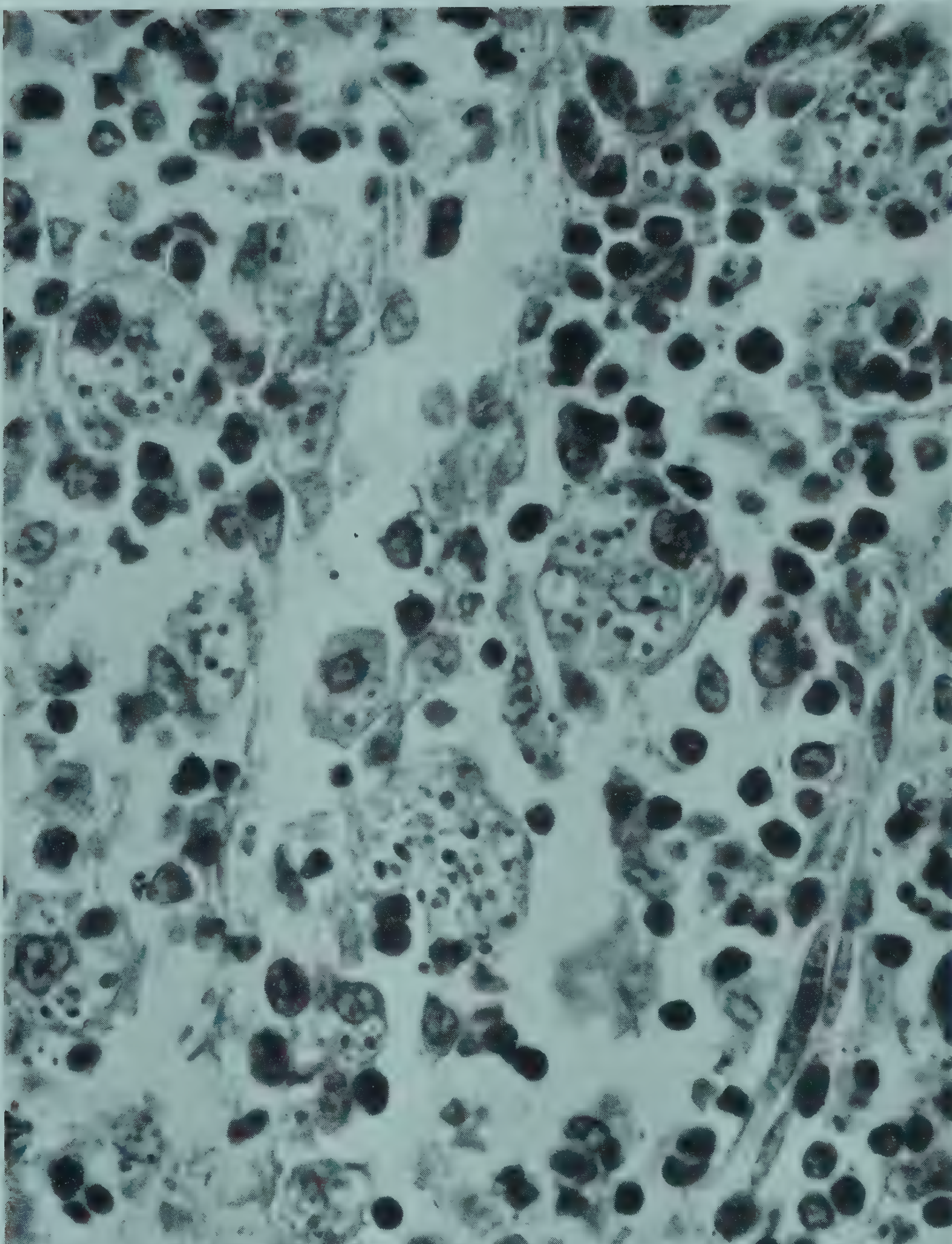

Fig. 3/2. Lymph gland in Histoplasmosis. From the same case as the preceding figure. There is close packing of the budding fungus bodies within the greatly enlarged macrophages. × 900. (*Photomicrograph kindly supplied by Dr. George J. Cunningham.*)

the injured red cells containing proliferating parasites are freely phagocyted by the reticulo-endothelial cells (see Taliaferro, 1949). In histological sections taken from the liver, spleen and bone marrow, many parasites can be recognized inside the macrophages lining blood channels. Ingestion of the parasites takes place more actively in individuals who have acquired some immunity or who have been treated with some anti-malarial drug, and it seems that under both these circumstances much of the destruction of the plasmodia is brought about in this way. The involvement of the reticulo-endothelial system in Leishmaniasis has long been known, and the examination of smears of the spleen or bone marrow for ingested Leishman-Donovan bodies has been extensively used for diagnostic purposes (see Shortt, 1947). Rey's (1943) experimental study of the events that follow the injection of a strain of the human pathogen *Leishmania braziliensis* into the tissues of susceptible animals has shown that the parasite undergoes rapid phagocytosis, first by the local tissue histiocytes and later by the macrophages and neutrophils that emigrate from nearby blood-vessels. Within a few hours of their ingestion, the micro-organisms show evidence of degenerative changes—a clear indication that the process is of protective value to the host. These observations on the defensive *rôle* of macrophages in this disease confirm and amplify those made many years ago by Meleney (1925) in a study of human and animal leishmaniasis.

Although histoplasmosis has been known for nearly half a century, it has only been within the last few years that its widespread distribution in both hemispheres has become recognized (Parsons and Zarafonetis, 1945). Much of the improvement in diagnosis has resulted from the use of laboratory investigations, and amongst these the examination of smears of sternal bone marrow has proved notably valuable (Pratt *et al.*, 1950). In a large proportion of cases of histoplasmosis, the causative fungus, *Histoplasma capsulatum*, can be recognized as small encapsulated organisms resembling Leishman-Donovan bodies in the cells of the reticulo-endothelial system (see Figs. 3/1 and 3/2) (Brandt, 1950). Indeed, it was this morphological resemblance that led to some early confusion in the differential diagnosis between histoplasmosis and leishmaniasis. How far the presence of the fungus within the macrophages can be interpreted as a defensive reaction on the part of the host is difficult to assess from human material alone ; an answer must await an experimental analysis of the course of the infection in a susceptible animal such as the

dog. The observation that macrophages are the only type of host cell that contains the parasite, however, is in itself material evidence that the removal of the latter from the circulating blood is a protective response and not a passive intracellular parasitization. Unfortunately, from the high case fatality rate for the disease, it is evident that any such defensive reaction is seldom successful.

Little is yet known of the part taken by the reticulo-endothelial system in defence against virus infections. The very small size of these pathogens has virtually precluded the application of the histological methods that have provided most of the evidence on the ways in which the tissues dispose of larger micro-organisms. The psittacosis virus, one of the largest of this class of organisms, has, however, been recognized in tissues examined by ordinary microscopical methods. Lillie (1930) studied the pathogenesis of the lesions of psittacosis, and found that the parasites could be seen in macrophages in many different organs. Again, it is necessary to make the same reservation as with histoplasmosis—it is difficult to distinguish between the colonization of host cells by the parasite and an active defensive response on the part of the reticulo-endothelial system to a viræmia. The case fatality rate in psittacosis, however, is notably less than that in histoplasmosis.

(*b*) *Formation of Antibodies.* The application of electrophoretic and other physico-chemical methods to the separation of the individual plasma proteins has shown that those globulins that possess the character of specific antibodies do not all fall into the same fraction. The large majority of these immune bodies are found in the category termed γ-globulin—a class of protein which in man is of moderately large size and migrates rather slowly in an electrical field. Some antibodies, however, separate in the more quickly moving beta fraction. Not only may the various antibodies formed in response to different antigens appear in different categories of globulins, but the same immunological specificity may be manifested by *two* electrophoretically separable proteins in the immune serum obtained from a *single* animal that has been immunized against a *single* antigen. Thus, Kekwick and Record (1941) have found that in the diphtheria antitoxic serum obtained from horses, the specific antitoxic character is associated with two separable globulins, one of the beta and one of the gamma type. Moreover, when two electrophoretically different types of specific antibody co-exist in the same serum,

one usually appears earlier in the course of immunization than the other, as though two distinct mechanisms with differing inertia existed in the body for their production. Often the two such antibodies differ in the degree of their specificity, that formed early in the course of immunization being more narrowly specific than that which appears later. Sometimes, too, they differ in their avidity for the antigen, so that one proves more effective for protective purposes than the other. The reason for drawing attention here to this modern work on the heterogeneity of even uni-specific antibodies is because it is a finding that must clearly be taken into account in any discussion on the source or sources of these immune bodies. It suggests in a manner that cannot be ignored that more than one type of host cell may be concerned in the formation of these specialized globulins.

There is much circumstantial evidence that, irrespective of whether or not other types of cell form antibodies, those of the reticulo-endothelial system take a major part in their production. In the first place, particulate antigens such as bacteria are removed from the circulation by those members of the system that line the blood sinuses in certain organs, and that the remains of these micro-organisms can subsequently be seen undergoing progressive dissolution in the cytoplasm of these cells. Nor is the removal of potential antigens by these cells confined to particulate matter, for Kruse and McMaster (1949) and Latta and his colleagues (1951) have recently shown that soluble proteins to which the strongly coloured dyes such as Evans blue, orange G and acriflavine had been firmly coupled by chemical linkage before injection could be seen to be removed from the plasma by the same system of cells and to accumulate in their cytoplasm as a dense pigment. In the second place, extracts made from the chief macrophage-containing organs of animals undergoing immunization have been found to contain specific antibodies before they become recognizable in the circulating blood. De Gara and Angevine (1943) injected rabbits intravenously with a single dose of a "concentrated" pneumococcal vaccine, and found that extracts made twelve days later from the liver, spleen and bone marrow, and, sometimes, from the regional lymph nodes, contained greater concentrations of the antibody than those from other organs or of the blood serum itself. These findings, together with a number of observations that specific antibodies accumulate in the medium of tissue cultures made from macrophage-containing organs of animals undergoing immunization at the time they are killed (Keuning

and van der Slikke, 1950) provide the main evidence for the belief that the reticulo-endothelial system is at least partly, if not wholly, responsible for the formation of these immune bodies.

The first indication that lymphadenoid structures are capable of forming antibodies was provided by McMaster and Hudack (1935), who found that after a subcutaneous inoculation of various vaccines made from members of the coli-typhoid group of bacteria, the corresponding antibodies first appeared in the regional lymph nodes of the area. These observations have since been extended by Burnet and Lush (1938), who found that the neutralizing antibody was notably high in the mediastinal lymph nodes of mice that had been inoculated intranasally with influenza virus, and by Ehrich and Harris (1942), who placed the matter on a firmer experimental foundation by cannulating the afferent and efferent lymphatic trunks of the popliteal lymph node in rabbits into whose feet antigens had been injected, and finding a substantially raised titre of the specific antibodies in the efferent lymph. Quite recently, this line of investigation has been further followed up by Oakley and his colleagues (1949), who have shown that after the subcutaneous injection of *alum-precipitated* diphtheria and tetanus toxoids into rabbits, guinea-pigs and horses, much larger amounts of the specific antitoxins could be recovered from the regional lymph nodes lying in the drainage area than from the homologous nodes on the opposite side. In ingeniously designed experiments in which alum-precipitated diphtheria toxoid was inoculated into one hind-leg of a horse and tetanus toxoid into the other, they showed that the raised local concentrations of antitoxins was due to their formation in the ipsilateral nodes and not merely to their accumulation in the nodal tissue fluids after escape from the blood circulating through the dilated capillaries of the inflamed nodes assayed.

While all the above experiments have yielded positive results upon the formation of antibodies in lymph nodes to which antigens are carried, Burnet and Fenner (1949) alone describe a negative finding in comparable experiments with staphylococcal toxoid. This failure, however, must not be regarded as throwing doubt on the findings with the other antigens. There are good reasons for believing that soluble proteins such as bacterial toxins and toxoids present a special case, for unless flocculated or precipitated by alum or in some way made particulate they possess sufficient diffusibility for much that is injected to escape from the site into nearby blood capillaries; as a result only a fraction of the inoculated

antigen ever reaches the local lymph nodes. Examination of the fate of suspensions after injection into the tissues have shown, on the other hand, that particulate matter is selectively removed by lymph channels, little if any entering the local blood capillaries (Drinker and Yoffey, 1941). So far, therefore, there is no material evidence to show that the cells of the lymph nodes can discriminate between particulate and soluble antigens and react differently accordingly.

The important part that the regional lymph nodes take in immunizations with alum-precipitated diphtheria and tetanus toxoids, according to Oakley's experiments, are of more than academic interest. If local lymph nodes can be forced to take a more important part in the production of antitoxins following prophylactic inoculations by replacing soluble formol toxoids with precipitated ones, it may be possible to enhance the immunity of poorly-reacting persons by multiplying the sites of injection in order to bring more nodes into production.

After a decade of discussion, the last few years have seen increasing agreement on the types of cell that form antibodies in lymphadenoid structures. Early work by Ehrich and Harris (1942) which suggested that lymphocytes were responsible has not been confirmed, and has since been retracted by both authors independently (Ehrich *et al.*, 1949 ; Harris and Harris, 1949), in favour of the plasma cell theory (see below). Similarly, the observation of Chase, White and Dougherty (1946) that the release of antibodies from lymphocytes is brought about by the adrenotropic hormone has not been supported by later and more detailed work (Eisen *et al.*, 1947). Recently support has come, first by Scandinavian and later by American investigators, for the view that plasma cells are concerned in some way with the release of both non-specific and specific antibody globulins. This belief is sustained by evidence from several sources. Firstly, cells resembling plasma cells, but of less mature appearance, accumulate in the spleen and lymphadenoid structures during the early stages of immunization, while later, when antibody production has reached its peak, mature plasma cells become notably conspicuous. Secondly, examination of plasma cells by Caspersson's microspectrographic technique has shown that the synthesis of proteins proceeds very actively in the cytoplasm of these cells. Thirdly, it has been shown on several occasions that patients with abnormally high concentrations of globulin in their plasma have an increase of plasma cells in their tissues, and it is well known that some of

the highest plasma globulin levels are found in patients with plasma cell myelomas.

It now seems possible that agreement will eventually be reached on the basis suggested by Fagraeus (1948): that reticulo-endothelial cells in such organs as the spleen may "produce antibodies, thereby developing into a type of cell with the morphological characteristics of plasma cells." Whether or not this forecast proves correct, it is clear that whatever type is primarily concerned with antibody formation, the cell responsible must be a long-lived one which is capable of retaining permanently the impress of a brief exposure to a single antigen. Otherwise, it would be difficult to account for the well-known finding that once an individual has been immunized with some antigen, he continues to possess for the rest of his life the capacity to react more promptly and energetically to a subsequent exposure to the same agent—a phenomenon which is known to immunologists as the "secondary response."

Disposal of Old Erythrocytes and Bile Pigment Formation

There is now general agreement that after their release from the bone marrow, erythrocytes survive in the circulating blood for roughly 120 days before they undergo disintegration. The evidence on which this belief is based is derived from a variety of sources which need not be considered here (Mollison, 1951). It will suffice to state that estimates of survival times that have been obtained from transfusions of serologically-distinctive, but compatible, homologous red corpuscles in man (Mollison, 1947), and with red corpuscles labelled either with the ^{15}N isotope of nitrogen (Shemin and Rittenberg, 1946), or the ^{14}C isotope of carbon (Bale *et al.*, 1949) in dogs, have all agreed remarkably well with older figures calculated from the rates of excretion of bile pigments. It may further be mentioned here that modern work indicates that all normal red corpuscles have a very uniform survival period—all that are released from the marrow at any particular time eventually disintegrate within a few days of one another. It is thus clear that erythrocytes are formed to last for a limited unvarying period only. Moreover, it seems likely that their final disruption depends more upon some agent that is inherent in the corpuscle itself than upon some extrinsic factor in the surrounding plasma, because red corpuscles from normal donors survive for the characteristic 120 days when transfused into patients with familial acholuric jaundice, whereas the

corpuscles of these latter survive for only twenty to thirty days in the blood-stream of normal persons.

How senile circulating red corpuscles are disposed of in a healthy person is still obscure. Once they become extravasated, erythrocytes are rapidly ingested by fixed macrophages, as Muir and Niven (1935) observed in subcutaneous tissues and Margarey (1951) in the lungs, and their subsequent disintegration is accompanied by the appearance of bile pigments and hæmosiderin in the cytoplasm of these cells. Formerly, it was widely believed, mainly on the basis of histological evidence, that the destruction of senile circulating red corpuscles was accomplished in much the same manner by the reticulo-endothelial cells of the liver, spleen and bone marrow. Doubt has been thrown on this, however, by some experiments made by Knisely (1936). Studying by transillumination the relation of reticulo-endothelial cells to erythrocytes in the living exteriorized spleen, he found that phagocytosis of the latter by the former does not take place in an undamaged organ, but that it occurs promptly after injury or in the agonal or post-mortem period. On these grounds, he was unwilling to accept erythrophagocytosis as a normal method of red cell destruction, and discounted its importance accordingly.

It is now known that the erythrocyte, although a dead blood element, Payling Wright and Arthur (1930) is not inert metabolically, but that it retains within its stroma a residue of enzymes from its formative period of development. Certain of these enzymes are concerned with maintaining the hæmoglobin in a form capable of reversible oxygenation and reduction, and so with preventing its undergoing progressive conversion into methæmoglobin—a pigment which is valueless for respiratory purposes (see Lemberg and Legge, 1949). Other enzymes catalyze further metabolic activities, whose nature need not be detailed here. Recently, however, Laser (1949) has extracted from the corpuscular stroma an ether-soluble substance which is actively hæmolytic, and it seems possible that the production of this substance inside the corpuscles during their survival time in the circulation may be concerned with their ultimate dissolution. The recognition of an ageing process in normal erythrocytes and their remarkably uniform " expectation of life " in the circulating blood, together with Knisely's failure to observe erythrophagocytosis in the normal living spleen, has lent support to the belief that the final disintegration of red corpuscles takes place in the blood itself and is independent of macrophage activity. Although

it has long been known that severe muscular exercise is often followed by hæmoglobinuria—the so-called " march hæmoglobinuria "—it has only recently been demonstrated by Gilligan and his colleagues (1943) that much milder grades of exertion are commonly succeeded by a significant hæmoglobinæmia. It seems likely that this effect of exercise is only an exaggeration of a hæmolytic process that is proceeding more or less continually in the normal body even at rest and is called forth by the greater mechanical stresses imposed by the more rapid circulation rate.

Shortly after the beginning of the last war, largely in consequence of the not infrequent transfusion accidents that resulted from the use of mis-matched blood, interest became revived in the fate of extracorpuscular hæmoglobin (see Hamilton Fairley, 1940). In the tropics, the question has long been of importance in connection with the blood crises of malaria, in which large numbers of erythrocytes are suddenly destroyed and their hæmoglobin liberated into the circulating plasma (Maegraith, 1948). There is now no doubt that such extracorpuscular hæmoglobin is treated by the body as a foreign substance, and it is believed that its subsequent destruction or elimination may follow one or more of three pathways which open up successively as the concentration of hæmoglobin in the plasma rises. At low concentrations, most of the hæmoglobin is removed by the reticulo-endothelial cells that line the blood-stream, and metabolized in the manner outlined below. At higher concentrations, hæmoglobin is broken down while still in the plasma, the hæmatin moiety released forming a compound termed methæmalbumin with the serum albumin. With still higher concentrations, hæmoglobinuria results. Since the reticulo-endothelial system is only known to be concerned with the first of these three modes of disposal, no further mention will here be made of the other two.

Once within reticulo-endothelial cells, hæmoglobin rapidly undergoes a series of destructive changes whose final products are bilirubin (now accepted as synonymous with hæmatoidin) and an iron-containing residue to which Neumann long ago gave the name " hæmosiderin." Largely through the work of Lemberg (the details of which may be found in his recent monograph with Legge), there is now considerable knowledge of the intermediary compounds that arise in this degradation process. For the present, it will suffice to state that the first change in the hæmoglobin molecule appears to be an oxidation in which, although the porphyrin still remains attached to both the iron and the

protein globin, the ring structure of this prosthetic group is broken open. This seemingly minor alteration suffices, however, to change completely the colour of the pigment, for the new substance, to which Lemberg has given the name "choleglobin," has a markedly greenish colour. At a later stage, the remnant of the now opened porphyrin ring is parted from the iron-globin complex to form first biliverdin, and later, by reduction, the common bile pigment, bilirubin.

The subsequent fate of bilirubin, after its escape from the reticulo-endothelial cells, is of importance in relation to the origin of jaundice, and the nature of the bile pigments that accumulate in the blood in its various forms. From the work of Martin (1949) on the electrophoresis of bilirubin in the presence of protein, it is now clear that this pigment readily forms complexes with albumin and α-globulin, but to no significant extent with any of the other plasma proteins. It is conceivable that those bilirubin-albumin and bilirubin-globulin complexes may be formed in different proportions in plasmas of differing protein composition, and that such differences may account for the "direct" and "indirect" forms of the van den Bergh reaction respectively. Modern work on the fractionation of plasma proteins, together with the recognition of their importance in forming "carrier-complexes" with smaller molecules, seems to have brought this much studied and difficult problem to the eve of solution (see Edsall, 1947).

Ferritin and Iron Storage. There has been increasing interest recently in the nature and fate of those iron-globin residues which are left behind in the reticulo-endothelial cells after separation of bile pigments. One important reason for this renewed attention has been the discovery of "ferritin." This is a protein substance found in quantity in extracts of the spleen, liver and bone marrow, and containing iron in the form of a ferric hydroxide to as much as 23 per cent. of its dry weight. The protein component of ferritin (apoferritin) is crystalline and has a striking avidity for iron. Its structure is that of a crystal lattice, the ferric hydroxide being held as micelles in its spaces, suggesting that the iron is easily liberated and readily available.

Studies with radio-active iron have considerably extended our knowledge of the significance of ferritin (see Michaelis, 1947; Hahn, 1948). The ferritin content of the reticulo-endothelial cell-containing organs rises after the administration of both hæmolytic drugs and iron salts, and in animals made suddenly

anæmic by bleeding, the deposits in the liver, spleen and bone marrow are utilized promptly during the course of blood regeneration.

The iron reaching the reticulo-endothelial organs and stored in them as ferritin is clearly derived from two sources—the inorganic iron absorbed from the intestine and organic iron derived from the destruction of senile red blood cells. In view of the avidity of apoferritin for iron it is probable that both fractions are in the first place stored in the liver, spleen, bone marrow and intestinal mucosa, and are later distributed from these depots to the erythropoietic marrow for hæmoglobin synthesis, to the muscles to maintain their myoglobin content, and to the tissues in general to be utilized in the synthesis of cytochromes, catalases and certain other intracellular enzymes. In the absence of abnormal destruction of blood inside the body, the labile and readily available iron stored in the reticulo-endothelial organs is almost entirely derived from the disposal of senile red blood cells, the daily iron loss by excretion by all routes being less than a milligram. Under physiological conditions, the amount of iron absorbed from the intestine *per diem* is of the same order, and it is impossible in normal animals materially to increase absorption even when an excess of ferrous iron in an ionizable form is given by mouth. Following blood loss, there is a prompt discharge of iron from the storage depots to the erythropoietic marrow (Haskins *et al.*, 1952), and, as the quantity of stored iron falls, the capacity of the intestinal mucosa to absorb iron becomes proportionately increased (Hahn *et al.*, 1943). The arresting fact that the rate of iron absorption is principally conditioned by the amount of iron stored in the body is fully attested by clinical experience, and an investigation of the distribution of ferritin in the tissues provides a clue to the mechanism by which this control is brought about. If an excess of iron is administered by mouth to normal guinea-pigs, large amounts of ferritin can be found in the mucosa of the small intestine. It is probable that this " ferritin barrier " in some way blocks further absorption of iron, while at the same time it forms an extraneous iron storage depot, for if the animals are now subjected to serious blood loss the intestinal ferritin is rapidly utilized for hæmoglobin synthesis. As the amount of iron held in the mucosa falls, the rate of absorption of inorganic iron from the intestine rises and the process continues actively until blood regeneration is completed. At this point, deposition of ferritin in

the mucosal cells recommences and the absorption of inorganic iron is again inhibited. This concept of the regulation of iron absorption has recently been reviewed by Granick (1949).

It is well established that iron in complex organic combinations is not absorbed to any significant extent from the intestine. In the three hæm-containing respiratory pigments—hæmoglobin, myoglobin and cytochrome—the metal is locked up in the tetrapyrrhol rings which the digestive juices are powerless to split open. On the other hand, when hæm iron is given intravenously in the form of hæmoglobin to anæmic dogs it is fully utilized, and in the hæmolytic anæmias most or all the iron set free as a degradation product of hæmoglobin is retained and re-used. It appears, therefore, that to split open their rings in the body these pigments must first be taken up by mesenchymal phagocytes and then broken down intracellularly by their enzymes.

Hæmosiderin has long been familiar to pathologists as a golden-yellow pigment rich in inorganic iron and easily demonstrated in the tissues by Perls' Prussian blue reaction. It consists of ferric hydroxide loosely combined with, or more probably adsorbed by, protein from which it can be readily separated by dilute mineral acids. Hæmosiderin and ferritin are clearly related chemically and biologically, but are probably not identical, for ferritin holds about 23 per cent. of its weight of iron, whereas, according to Granick, the iron content of hæmosiderin is about 35 per cent. Furthermore, it is impossible to demonstrate the presence of hæmosiderin by Perls's reaction in the reticulo-endothelial organs under strictly physiological conditions, although it is reasonably certain that they normally hold an appreciable quantity of stored ferritin. Granick suggests that hæmosiderin may be formed under conditions, generally of a pathological nature, in which ferric hydroxide is being formed particularly rapidly, with the result that the lattices in the apoferritin crystals become more highly charged than usual with this material. It is usually agreed, however, that the term "hæmosiderin" covers a group of kindred substances, and that several compounds carrying large but variable amounts of iron are included in the group of hæmoglobin disintegration products which give a positive Perls' test.

Disorders of Metabolism and Storage of Lipoids

Apart from localized and generalized xanthomatous deposits, in which macrophages become distended with excessive quantities

of cholesterol, the reticulo-endothelial system becomes extensively involved in two interesting disturbances of lipoid metabolism. In both the uncommon familial Gaucher's disease and in the still more rare Niemann-Pick's disease, the cells of this system which line both blood and lymph sinuses present a striking appearance; their numbers increase and their cytoplasm becomes grossly swollen and foamy with innumerable droplets of particular lipoids.

In Gaucher's disease this material is now known to be kerasin, a cerebroside in which a carbohydrate is united to an ester of the base sphingosine and the fatty acid lignoceric acid. This ester, which Thannhauser (1950) has termed "ceramide," is interesting because it again figures as a part of the abnormal lipoid in the kindred Niemann-Pick's disease, and may thus occupy some central place in both these metabolic disturbances. Cerebrosides have long been known as extractives of nervous tissues and to a lesser extent of other organs, and it has long been assumed that the lipoid deposited in the liver, spleen and bone marrow in Gaucher's disease was of the same chemical constitution. Accordingly, it was generally believed that these deposits were laid down because of some metabolic disturbance in which excessive quantities of kerasin was produced, and that the resulting high concentration of this substance in the blood led to its ingestion and storage by this group of vascular macrophages.

Several facts have recently emerged which render this explanation of the pathogenesis of Gaucher's disease less likely, and which indicate that tissue macrophages may take a more active and specialized *rôle* in lipoid metabolism than has been formerly assumed. The first of these findings was the discovery that there is an important difference in the constitution of the cerebrosides extractable from the brain and those obtainable from the macrophage-containing organs in this disease. Whereas in the former the carbohydrate residue is galactose, in the latter Halliday and his colleagues have recovered glucose (1940), and this major difference between the two cerebrosides has since been confirmed by others. Further, the finding of Ottenstein, Schmidt and Thannhauser (1948) that the kerasin present in the red cell envelopes in patients with Gaucher's disease is a galactoside, seems to eliminate the possibility that the macrophage deposits are accumulation of undigested lipoids from the stroma of broken-down red cells. Lastly, estimations of the concentrations of cerebrosides in the plasma of patients with Gaucher's disease have

failed to support the primary assumption of the deposition theory, namely, that these substances are present there in excessive amounts. To account for these more recent findings, Thannhauser (1950) has suggested that the essential disturbance in these two macrophage lipoidoses lies in the intracellular enzyme systems of these cells, especially that concerned with the metabolism of the ceramide constituent of the lipoid. The test of this hypothesis hinges chiefly on whether or not Thannhauser's failure to find excessive concentrations of cerebrosides in the blood of patients with Gaucher's disease can be confirmed by other investigators. Irrespective of whether or not Thannhauser's contention is correct, however, it should be pointed out that it is possible to reproduce experimentally in animals a condition which morphologically at least closely resembles Gaucher's disease. When emulsions of purified cerebrosides were injected into rabbits by Christianson (1941), foamy cells identical in appearance with Gaucher cells appeared in the liver, spleen and lymph nodes. It is thus evident that if cerebrosides are present in abnormally high concentration in the plasma, these macrophages can selectively remove and store them. It would be of interest to allow such animals to survive for a longer period than Christianson did, in order to see whether these swollen cells were capable of metabolizing the lipoid and reverting to their normal appearance.

In the still less common Niemann-Pick's disease of infants, the reticulo-endothelial system in the liver, spleen, bone marrow and lymph nodes again shows the typical foamy transformation of cells. In this disease the lipoid that accumulates is not lecithin, however, as was formerly believed, but sphingomyelin, a substance that resembles the cerebrosides in being an important extractive of the central nervous system. Further, it has a resemblance to the cerebrosides in chemical constitution, being formed by the combination of ceramide and phosphorylcholine instead of ceramide and a hexose carbohydrate. But whereas the cerebroside recoverable from the diseased organs in Gaucher's disease is typically the abnormal glucoside of ceramide, there is as yet no indication that the sphingomyelin of Niemann-Pick's disease has any different composition from that of normal organs. The two diseases, however, appear to resemble one another in the absence of any rise in the concentration of the particular lipoid in the circulating blood.

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CHAPTER IV

RETICULOSIS AND RETICULO-SARCOMA

During the whole of the post-natal period immature mesenchyme retains its pre-natal capacity to proliferate to a far greater degree than any other tissue, and responds by progressive hyperplasia to a far wider variety of stimuli. Whilst its highly reactive, fertile and multipotent "blast" cells are scattered throughout all tissues, they are so heavily concentrated in the reticulo-endothelial organs that lymph-node enlargement, splenomegaly, hepatomegaly and bone marrow involvement due to progressive proliferation of immature mesenchymal cells is the common morbid anatomical background to a vast number of ætiologically unrelated diseases. When the stimulus to proliferation is infection or a disorder of intermediate lipoid metabolism, a large proportion of the proliferating cells differentiate to produce mobile mononuclear phagocytes, and the swelling of the reticular organs is largely the result of close packing of the pulp and the vascular and lymphatic vessels by well-differentiated histiocytes. In the leukæmias and primary erythræmias there is apparently purposeless proliferation of immature blood cells in the marrow and, by "myeloid metaplasia" in the spleen and liver, and often in the lymph nodes. When all the reticulo-endothelial organs are enlarged at the same time as a result of progressive cellular proliferation, the process is said to be systematized. Systematization may develop quickly in some of the prolonged acute infections, such as brucellosis and infective mononucleosis. It is clinically outspoken in visceral leishmaniasis, American trypanosomiasis and histoplasmosis. In a far larger variety of infections, systematized proliferation follows or accompanies one or more inflammatory reactions in non-reticular tissues. This occurs, for instance, in congenital syphilis. Systematization may be delayed for long periods, the proliferation being confined to one component of the lympho-reticular tissues, frequently the lymph nodes.

Hodgkin's disease may be taken as representative of another group in which systematized reticulo-endothelial proliferation forms a common pathological background. The cellular proliferation and differentiation in these diseases is invariably complex

and a confusing variety of labile cytological pictures is encountered in the swollen organs. Disregarding the difficulties which these structural variations entail, two basic facts emerge. First, that progressive proliferation of immature mesenchyme is the cause of the lymphadenopathy and splenomegaly, and second, that in all the diseases of this group we are in a state of total ignorance with regard to their ætiology. So fruitless has the long search for an ætiological agent proved to be that there is now a growing tendency to regard the diseases of the Hodgkin group as being neoplastic. Against this it may be pointed out that indiscriminate destructive neoplastic infiltration of non-rectiular tissues does not usually occur in these conditions, and the clinical consequences are largely the result of pressure by the enlarged glands and organs on important or vital structures. It must be agreed, however, that so long as this profound ignorance of ætiology continues we must admit that the cytology of the lesion in Hodgkin's disease and allied conditions possesses several of the basic characters of the neoplasia. In this connection serious consideration must be given to the fact that the tempo of the cellular proliferation shows a striking degree of variation not only from one case to another, but during the course of a single case. It may extend over years of chronic illness at one end of the scale, terminate in a year or so at the other, or become frankly sarcomatous in a small but significant number. At the present time all diseases of the Hodgkin group are collected together by American pathologists as " malignant lymphomata," and Willis (1948) follows the American classification. In this country the old belief that Hodgkin's disease is infective in origin dies hard, in spite of the fruitless search for an ætiological agent. To express our uncertainty and at the same time our unwillingness to regard these diseases as neoplastic, it has become customary to refer to all the progressive proliferative diseases of the reticular organs whose ætiology is completely unknown as *the reticuloses.* This practice is illogical and dangerous unless certain assumptions are made, and an attempt will be made to examine it in the light of recent investigations.

About a quarter of a century ago it was suggested by Letterer that progressive systematized proliferation of the immature mesenchyme of the reticular organs was so common a general pathological process that every disease in which this occurred should be called a reticulosis. It must be clearly understood that at this time the term " reticulosis " had little more significance

than the word fibrosis has now ; it was used as a convenient label to describe a tissue reaction common to a multitude of diseases. Some reticuloses, and these comprise the large majority, were then described as infective ; the lipoid storage diseases became lipoid reticuloses, and the leukæmias, hæmic reticuloses. It is, for instance, pathologically correct to describe typhoid fever as an infective reticulosis, and the well-defined clinical entity chronic lymphatic leukæmia can be placed under a common pathological umbrella with all other leukæmias as a hæmic reticulosis, just as asbestosis can be grouped with many other diseases of a totally different ætiology as pulmonary fibrosis. In 1932, Pullinger proposed that as Hodgkin's disease fell by definition within the reticulosis group it could be defined pathologically as a "fibro-myeloid" reticulosis, in view of the fact that the proliferating mesenchyme in the rèticular organs strongly tended to differentiate into fibre-producing fibroblasts and granular leucocytes. This proposal led some observers to envisage the possibility that *a cytological picture determined by the degree and type of differentiation of the proliferating mesenchyme in a lymph node, the spleen, or bone marrow, might determine, be intimately related to, or could be broadly but accurately correlated with the natural history of a disease with a fairly well-defined clinical picture.* This possibility was investigated and strongly upheld by Robb-Smith. In his thesis submitted for the Doctorate of Medicine in the University of London in 1936 the correlation was described in a clinical and pathological survey, which at that time was clearly too limited to be conclusive. He has more recently upheld his claim, basing it on extensive experience, and now maintains (1947) that when the infective, lipoid and hæmic reticuloses are excluded, there remains a group of seven diseases in each of which the cytological pattern of the proliferating mesenchyme can be correlated with the natural history and clinical features of a specific disease entity. The *diseases* he describes are :

1. Lymphoid follicular reticulosis.
2. Lipomelanic reticulosis.
3. Lympho-histiocytic medullary reticulosis.
4. Lympho-reticular medullary reticulosis.
5. Reticulum-celled medullary reticulosis.
6. Histiocytic medullary reticulosis.
7. Hodgkin's disease (fibro-myeloid reticulosis of Pullinger).

Each of these varieties of reticulosis of unknown ætiology is given a clear-cut title which describes not only a cellular reaction,

but, having been correlated with incidence, mean age at onset, clinical features and survival rate, becomes the name of a specific clinical entity. In the case of lymphoid follicular reticulosis (giant follicular lymphoma) and fibromyeloid reticulosis (Hodgkin's disease) it would be possible to find a fair measure of agreement amongst pathologists and clinicians that the cytology of the affected organs can, with certain reservations, be broadly correlated with the manifestations of a disease having its own clinical individuality. There is not at the present time the same measure of clinical and pathological agreement with regard to the other reticuloses in this group. There is one obvious reason for this. With the exception of giant follicular lymphoma (lymphoid follicular reticulosis) and Hodgkin's disease, the remaining diseases are relatively rare, and a long period may elapse before Robb-Smith's claims are confirmed or refuted by other observers. Another fact which has emerged from recent enquiries is that the histological pattern of the reacting organs is remarkably unstable and varies from time to time in the same individual in a most confusing fashion. Finally, there is the undoubted tendency for frank malignancy to supervene. The protagonists of the neoplastic theory regard this as nothing more than the assumption of anaplasia by a new growth which for a long period has exhibited the clinical and pathological features of low-grade malignancy.

In previous editions of this book we have, in justice to Robb-Smith's pioneer efforts in this field, reported his main conclusions, believing that adequate clinical and pathological confirmation or refutation of his claims would eventually be published. This has not materialized and until we are on surer ground we believe that the word "reticulosis," if used at all, should be employed exclusively to describe a cellular reaction or a cytological picture in the reticulo-endothelial organs. It is clearly inaccurate and misleading, in our present state of uncertainty, to use the word to describe a specific disease.

G. HADFIELD.
C. V. HARRISON.

Giant Follicular Lymphoma (Follicular Lymphoblastoma or Lymphoid Follicular Reticulosis). This is a relatively benign disease and forms about 5 per cent. of this group of progressive lymphadenopathies. It affects both sexes equally and its main incidence falls in the fifth decade. The majority of patients

survive more than five years. The lymph nodes are chiefly involved and may become very large ; the three commonest sites are the cervical, retroperitoneal and inguinal groups. The spleen is enlarged in about 40 per cent. of cases. There is no anæmia or other blood change and no involvement of the bone marrow, tonsils or internal organs. There may occasionally be involvement of the lachrymal gland. The disease responds rapidly to irradiation, but its course is unpredictable. A few patients die of the disease itself and they usually show a greatly enlarged spleen crowded with enlarged malpighian bodies, and serous effusions due to the pressure of enlarged nodes on vessels. Some patients die of intercurrent disease, but in the majority the disease progresses into some more malignant form. In Jackson and Parker's series of 39 cases, 14 developed lymphosarcoma and 11 developed other forms of "malignant lymphoma."

Histologically the affected nodes consist of closely packed lymphoid follicles which compress the residual tissue into narrow strands (see Fig. 4/1). This pattern can often be recognized in the gross specimen, and is clearly seen in silver impregnated sections with low magnification because the follicles are virtually devoid of reticulin and are outlined by the compressed reticulin of the original tissue. The individual follicles consist of a large central germinal follicle composed of lymphoblasts surrounded by a narrow band of packed lymphocytes. A crack frequently occurs between these two parts even in well-prepared sections. The cells of the germinal centres are remarkably uniform and there are no large macrophages among them. Mitoses are present but infrequent. The capsule of the gland is not invaded. Giant follicular lymphoma bears a superficial resemblance to a chronic inflammatory follicular reaction, but can be distinguished by the absence of macrophages in the germinal centre, the paucity of mitoses, the absence of an accompanying sinus reaction, and the extreme crowding together of the large follicles.

Hodgkin's Disease. Universally regarded by clinicians as a well-defined clinical entity, this disease constitutes about 30 to 35 per cent. of progressive lymphadenopathies. It affects males about two and a half times as often as females and can occur at all ages, though it has a peak incidence in the fourth decade. The lymph nodes are the main site of involvement in over 90 per cent. of cases ; the spleen is involved in about half the cases, internal organs (liver, lungs, kidneys) in about 10 per cent., and the bone marrow in about 20 per cent. In the later stages there

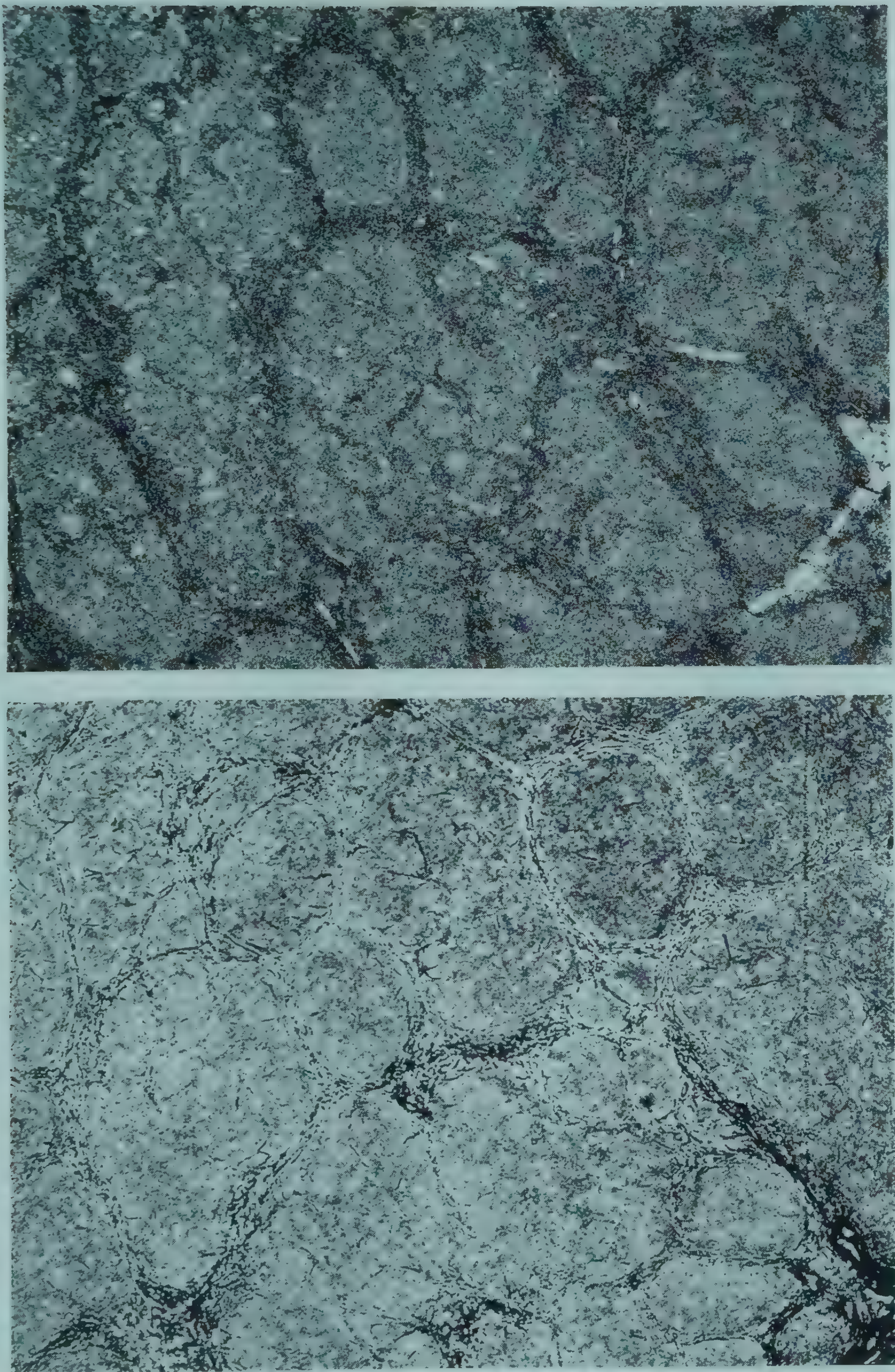

FIG. 4·1. Giant Follicular Lymphoma. *Above :* Tightly packed, enlarged follicles replacing normal node. (Stained hæmatoxylin and eosin. × 18.) *Below :* Silver impregnation showing follicles devoid of reticulin with compressed reticulin fibrils between. × 18.

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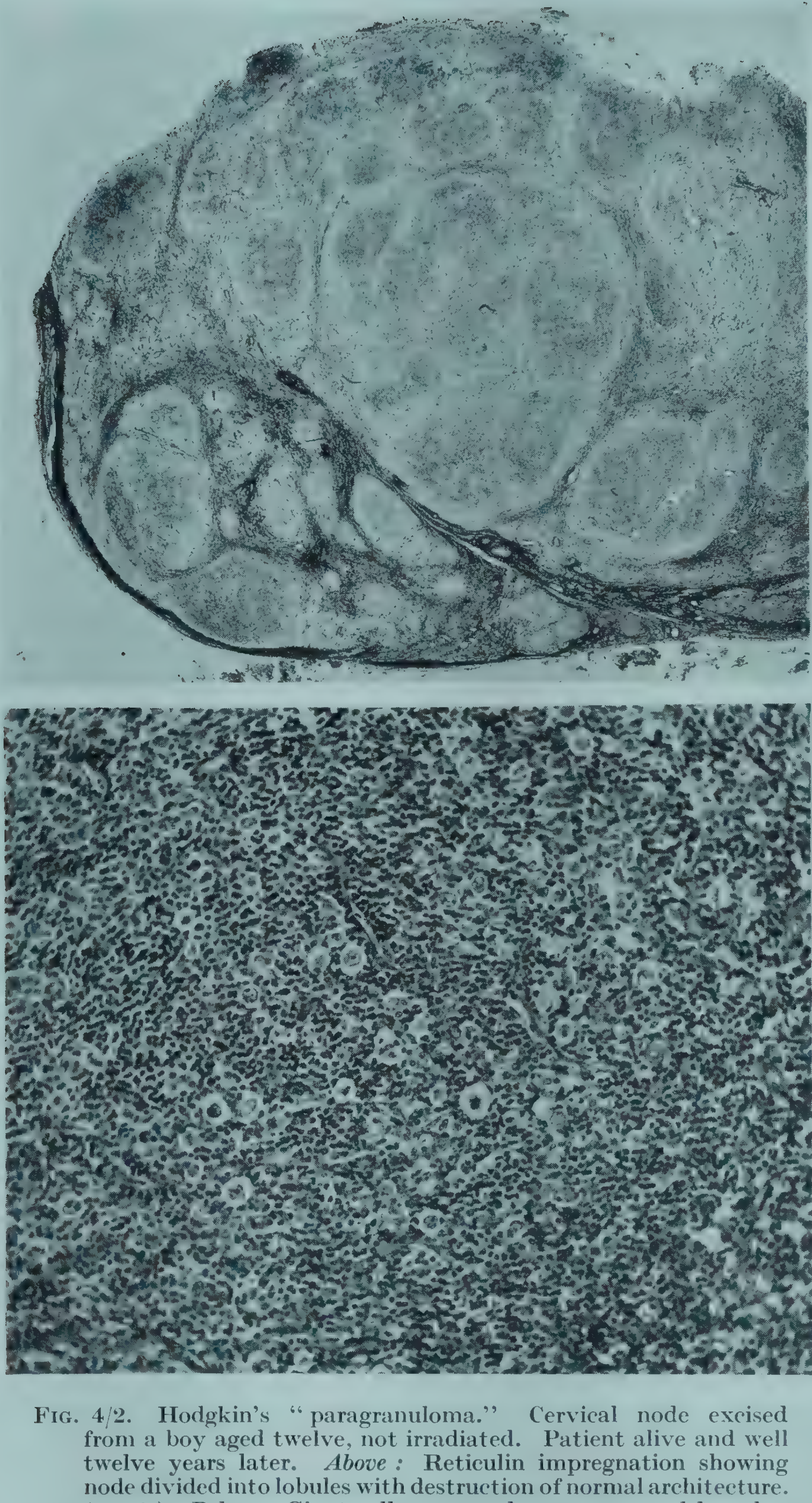

FIG. 4/2. Hodgkin's "paragranuloma." Cervical node excised from a boy aged twelve, not irradiated. Patient alive and well twelve years later. *Above :* Reticulin impregnation showing node divided into lobules with destruction of normal architecture. (× 18.) *Below :* Giant cells scattered among normal lymphocytes. (Stained hæmatoxylin and eosin. × 175.)

may be a hypochromic anæmia, but abnormal cells are not seen in the blood and there is no characteristic blood finding. The disease runs a rather prolonged course with a mean survival of about four years, though treatment may modify this considerably.

The histology of the lesions is essentially variable. The glandular architecture is totally destroyed and replaced by a diffuse overgrowth of abnormal pleomorphic reticulum cells. Scattered amongst these is a variable number of Hodgkin's giant cells. Mitoses may be seen in the reticulum cells or in the giant cells, but are normally scanty. Eosinophils, plasma cells, polymorphonuclears and lymphocytes occur in variable numbers; the amount of reticulin varies, tending to be scanty in early or in more rapidly growing lesions, and in slow growing cases of longer duration forming a dense tangled network which progresses into thick strands of collagen. Necrosis may occur in late lesions.

The clinical course of Hodgkin's disease varies widely. Some cases run a peculiarly benign course over many years, others have a rapid course with early death. Attempts have been made to correlate differences of structure with this difference of behaviour. Jackson and Parker (1947) have separated their benign cases under the title "Hodgkin's paragranuloma." In their series there were 41 such cases compared with 237 of classical Hodgkin's disease—an incidence of 1 : 6. The disease affected males three times as often as females and had its main onset in the fourth decade. The lymph nodes were enlarged, but other tissues were not affected. Most of the patients have remained alive and well for a period of many years, usually following radiotherapy. Of 12 cases who died, 5 died of coincidental disease, and in the other 7 the condition had changed into classical Hodgkin's disease.

At present it is difficult to assess the significance of this as a separate sub-group, partly because the published descriptions are brief and partly because the behaviour of Hodgkin's disease is dependent on treatment. Peters (1950) recently reviewed 113 cases of Hodgkin's disease which were all subjected to biopsy and classified according to the criteria of Jackson and Parker. They were all subsequently treated by irradiation and followed for a number of years. It was found that taking all cases together, the average five-year survival was 51 per cent., and ten-year survival was 35 per cent.—results which indicate that treatment can greatly modify survival rates. Although cases of "paragranuloma" survived on the average longer than cases of classical Hodgkin's disease, their survival rate was no better than that in

classical cases having the same degree of involvement at the onset of treatment. The cases of " paragranuloma " only survived longer because they had fewer glands involved at the commencement of treatment. Whether or not this variant of Hodgkin's disease proves to have a clinically distinct behaviour, it has a distinctive morphology that needs to be recognized in order to avoid confusion.

Histologically, the normal architecture of the node in this " paragranuloma " group is totally destroyed, though occasionally a fragment of normal tissue may survive. The capsule is slightly thickened and there are a few strands of collagen or closely-packed reticulin fibrils traversing the node and tending to divide it into lobules. Within these lobules there are scanty short fragments of reticulin. The node consists almost entirely of well-formed lymphocytes and is devoid of any architectural pattern. Among the lymphocytes are relatively numerous Hodgkin's giant cells (see Fig. 4/2). The pleomorphic reticulum cells that characterize Hodgkin's disease are absent or very scanty. Eosinophils, polymorphonuclears and plasma cells can usually be found, but are present in small numbers. These cases can be distinguished from typical Hodgkin's disease by the absence of the pleomorphic reticulum cells and the scanty reticulin. They can be distinguished from lymphosarcoma by the presence of the giant cells, the absence of mitosis and the absence of capsular invasion.

At present it seems highly desirable that such cases should be distinguished and carefully followed in order to establish the extent that their behaviour differs from that of typical Hodgkin's disease.

Another variant of Hodgkin's disease is that which runs a more rapidly fatal course. Many workers have tried to distinguish such cases under the title Hodgkin's sarcoma or malignant Hodgkin's disease. Such cases have been found to occur about a decade later than typical Hodgkin's disease and to affect women as often as men. The marrow and internal organs are affected more often, but splenomegaly is less common.

Histologically these cases differ from typical Hodgkin's disease only quantitatively. There is more active proliferation of pleomorphic reticulum cells, mitoses being more frequent, giant cells are fewer, and there is less infiltration with eosinophils and polymorphonuclears. Reticulin fibrils are relatively scanty and the capsule of the gland is often invaded. Although these various

differences suggest more active growth and can be correlated with a shorter average survival, a study of a series of cases shows that there is in fact every gradation between typical Hodgkin's disease and the so-called Hodgkin's sarcoma. Furthermore, since individual cases may show similar differences between one group of glands and another, it does not seem justifiable to separate a special sub-group on purely quantitative histological criteria.

Lymphosarcoma. Lymphosarcoma has approximately the same frequency as Hodgkin's disease and similarly affects men twice as often as women. Though occurring at any age, it has a peak incidence in the fifth decade. It may begin at any site where there is reticulo-endothelial tissue, but most often affects lymph nodes. The spleen is enlarged in about half the cases and other internal viscera in about 20 per cent. The bone marrow shows gross deposits in about 25 per cent., but microscopic infiltration is much more frequent. The disease runs a frankly malignant course and produces widespread metastases, but in the average case the majority of the deposits occur in reticulo-endothelial tissues. The lymphoid tissue of the stomach and intestine is often involved and so are the kidneys. The average survival in published series is from two to three years.

Histologically the lymph node architecture is totally destroyed by a uniform structureless overgrowth of lymphoid cells which invade the capsule and adjacent fat. Reticulin fibrils are scanty. The tumour cells vary from case to case. In some they are well-differentiated lymphocytes, in others they resemble lymphoblasts, being about 15μ in diameter with a narrow rim of faintly basophilic cytoplasm around a central vesicular nucleus with a sharp nuclear membrane and a scanty fine chromatin network. Nucleoli are infrequent, but mitoses are numerous though they are often difficult to define among darkly-staining nuclei. Scattered primitive reticulum cells can frequently be seen among the lymphocytes and can be recognized by their larger size and paler staining. Many workers have separated a lymphoblastic from a lymphocytic type on histological grounds, but these types cannot be correlated with any significant differences in behaviour, though the better differentiated cases tend to have a slightly longer survival. In practice, however, it is impossible to isolate two clear-cut histological groups and it appears that there are two extremes with infinite gradations between them, and since there is no constant difference in behaviour the distinction is a rather arbitrary one.

Blood Changes in Lymphosarcoma. The question whether lymphosarcoma and lymphatic leukæmia are essentially different or a part of the same process is a matter of debate. At either end of the scale the two diseases are distinct clinical entities, but there is a very large number of cases in which a clear distinction is not possible. The mere presence of abnormal lymphoid cells in the circulating blood is not an adequate definition because a considerable number of cases show the characters of lymphosarcoma for a long time before developing a leukæmic blood picture. Equally the presence of tissue invasion does not exclude a leukæmic blood picture. Histologically neither the pattern of the tissues nor the cell cytology assists in making a distinction, though the presence of leukæmic blood can, of course, be recognized in tissue sections. At present one can only say that, although two typical disease entities can be defined, the overlap is sufficient to suggest that they may well prove to be variants of one basic process. The temporary but astonishing clinical improvement that follows the treatment of lymphosarcoma by the adrenocorticotrophic hormone of the adeno-hypophysis is as yet totally unexplained.

Reticulo-sarcoma is about half as frequent as either lymphosarcoma or Hodgkin's disease. It affects both sexes equally and has a peak incidence in the fifth and sixth decades. The average survival is about two years. In most cases the lymph nodes are the main site, but in a significant number of cases the internal organs are primarily affected and the lymph nodes are not greatly enlarged. The spleen is enlarged in about 25 per cent. and the bone marrow contains gross deposits in 10 to 20 per cent. of cases.

Histologically the lymph nodes are totally replaced by an overgrowth of malignant reticulum cells of uniform morphology ; they tend to destroy the capsule and invade surrounding tissue. The tumour cells may occur as a syncytium or more often as separate cells which are fairly large (25μ) and have a pale staining cytoplasm and a rounded nucleus with a scanty chromatin network and a prominent nucleolus. In some cases the cells resemble histiocytes and may be oval in shape with an indented nucleus. Reticulin fibrils are scanty in the least differentiated types and a little more plentiful when differentiation is more advanced ; they are, however, usually scanty compared with Hodgkin's disease. In a small proportion of cases the cells are frankly pleomorphic.

Various attempts have been made to sub-divide the reticulo-sarcomata according to their cytology or their reticulin production, but no clear difference of behaviour can be correlated with these subdivisions, nor are they clear-cut histological entities. As in the case of the lymphosarcomata there is an infinite series of transitions between different types.

There are no characteristic blood changes in reticulo-sarcoma. In the later stages of the disease most cases show a hypochromic anæmia and in a few cases abnormal monocyte-like cells may escape into the circulation, though rarely in large numbers.

Uniformity of Histological Types

The above descriptions, which are essentially histological, depend for their validity upon the assumption that the different types of lesion are clearly recognizable entities and that they breed true during the evolution of the disease in any one patient. These assumptions call for close scrutiny. If a large series of cases is examined, it will be found that the vast majority can readily be classified into the types described. There will, however, always remain a small proportion that shows a histological pattern intermediate between the recognized types. In particular, cases occur that appear to be intermediate between reticulo-sarcoma and lymphosarcoma, between Hodgkin's disease and reticulo-sarcoma, and between giant follicular lymphoma and lymphosarcoma. Such cases cause practical difficulties of diagnosis and are probably of significance as indications that the lesions described are different examples of one underlying process rather than different disease entities. This view receives greater support from a study of the constancy of histological type in individual patients. Gall and Mallory (1942) found that of 56 patients subjected to two or more biopsies 13 showed an appreciable difference between the two histological patterns. Of recent years, with the increasing use of biopsy, more examples of such change have been reported. Herbut *et al.* (1945) recorded 6 cases in which complete change of histology occurred from Hodgkin's disease to reticulo-sarcoma, or the reverse. Similar cases have been reported by Symmers (1948), but the most convincing evidence has been advanced by Custer and Bernhard (1948) in a study of 700 cases of Hodgkin's disease and 600 cases of other progressive lymphadenopathies from the American Army Institute of Pathology. Among the 700 cases of Hodgkin's disease there were 138 with biopsy and autopsy material, and 69 with two or

more biopsies. Of the former, 39 per cent., and of the latter, 31 per cent., showed a wide enough divergence of histological pattern to necessitate reclassification. In addition, the autopsy cases frequently showed an equally wide divergence between different sites. Similar variations were also noted in the case of other lymphadenopathies, especially giant follicular lymphoma, which rarely ran its course without undergoing transformation to some more malignant type. In general, cases tended to progress towards greater malignancy though this was not invariable. These observations are of great interest because they indicate an underlying unity in this group of diseases. They are also a warning against any too rigid system of classification based on purely histological criteria. Nevertheless, broad histological classification is clearly of value in separating conditions which have different prognoses. Where histological diagnosis is of the greatest value is in deciding whether a lesion is progressive or merely inflammatory ; here the two most important criteria are the loss of the basic architecture of the node and the presence of abnormal and frankly immature cells.

Myeloma or plasmacytoma can occur in three forms—as the classical myelomatosis, as solitary myeloma, or as extra-osseous myeloma, the latter two being rare. Myelomatosis occurs between the ages of forty and seventy, with a peak incidence at fifty-five, and affects men at least twice as often as women. It may present in various ways, but the commonest are bone pains, obscure anæmia and spontaneous fracture. Bone pains are common, often severe, and particularly affect the back. Anæmia of hypochromic or of leuco-erythroblastic type may be the first sign ; it is usually severe and is found in 75 per cent. of cases. Pathological fracture is very common and may be the first sign, and the ribs are affected rather than weight-bearing bones. The tumour occurs as focal deposits in the bones containing red marrow. The sites most often involved, in order of frequency, are spine, ribs, skull, sternum, pelvis and proximal part of limbs (Geschickter and Copeland, 1949). In most cases the tumour excites local osteoclastic absorption of bone so that radiographs show clear, punched-out foci of translucency. In early cases, however, the tumour may be confined to the normal marrow spaces, and radiographs will then be negative. In addition to the neoplastic foci, however, there is a generalized increase of plasma cells throughout the marrow, and in practically all cases a diagnosis can be made on marrow puncture, the film showing an

increased proportion of plasma cells and more particularly the presence of immature or morphologically abnormal forms (Fig. 4/3).

In most cases of myelomatosis there are changes in the plasma proteins. The total proteins are increased, sometimes to values as high as 10 per cent. This increase may be in the gamma globulin or may be due to the presence of abnormal globulins, some of which crystallize out spontaneously in the cold. If abnormal globulins are present the electrophoretic pattern of the serum may show an abnormal peak near the beta globulin or between it and the gamma globulin. The relation between the abnormal plasma protein and the Bence-Jones protein in the urine is not clear. In some cases they appear to be identical, in other cases they are different, and in general there is no clear correlation between them (Marrack and Hoch, 1949).

Considerable work has been done on the nature of the Bence-Jones protein (Devine, 1941 ; Marrack and Hoch, 1949), and it appears that it is not a single entity but a group of proteins having a similar constitution and similar reactions. Dent and Rose (1949) recently studied a case and have shown that the Bence-Jones protein is devoid of methionine, a character in which it differs from most animal proteins and resembles those of certain viruses. The source of the abnormal protein is uncertain, but is believed to be the plasma cells. There is evidence that normal plasma cells are the source of gamma globulins (Teilum, 1948), and it would not be surprising if neoplastic plasma cells secreted abnormal forms of globulin.

Closely related to the production of abnormal blood and urinary protein in cases of myelomatosis is the occurrence of amyloid deposits. Chemically, amyloid consists of a combination of an acid carbohydrate (chondroitin sulphuric acid) with globulin. In myelomatosis it is probable that the globulin component is derived from the abnormal circulating globulins. Amyloid deposits in myelomatosis are not infrequent and tend to occur in unusual situations, such as skin, heart and tongue, leaving the liver, spleen and kidney relatively unaffected. This form of amyloid appears to differ from the classical form in frequently giving anomalous histo-chemical reactions, presumably because of a different chemical constitution, and it is sometimes designated para-amyloid.

Myelomatosis runs a malignant course with an average survival of about three years. Death may be due to amyloid disease,

to renal failure due to the deposition of protein casts in the tubules, to progressive anæmia, or to cachexia and terminal infection. At autopsy metastases may be found in lymph nodes, spleen or liver, but not elsewhere.

Histologically the tumours consist of sheets of plasma cells supported by a minimal amount of fine vascular stroma. The individual cells vary from case to case, in some they are typical differentiated plasma cells, in others they may be so anaplastic as to make recognition extremely difficult Cases of myelomatosis have been recorded in which the tumour cells were believed to be other than plasma cells, but such instances are rare and there is always the possibility that the difficulty of recognition is due to a marked degree of anaplasia

Solitary myeloma is a localized form of the disease in which one large tumour is present without generalized marrow involvement. The condition can be suspected in cases presenting with a localized tumour, but it is impossible to be certain that the disease is solitary unless surgical excision is followed by survival for several years or complete autopsy, including microscopy of many samples of marrow, fails to reveal other deposits.

Extramedullary myeloma is relatively uncommon. Jackson and Parker encountered 5 amongst 62 cases of osseous myeloma. Hellwig collected 128 cases from the literature up to 1943. In about half the recorded cases the tumour has arisen in the nose, pharynx or larynx. Most of the others are primary in the conjunctiva. This latter group is probably open to doubt because of the difficulty in distinguishing a true plasma cell tumour from a plasma cell granuloma, and the fact that in only 2 cases did the lesions recur. This criticism, however, does not apply to the cases originating in the respiratory tract, or to the few cases recorded as originating in glands or other internal organs, because in a significant proportion of cases the disease was not cured by surgical excision and later generalized throughout the skeleton, producing the typical picture of myelomatosis. Hellwig draws attention to the observation that the degree of cytological differentiation is not a reliable guide to prognosis because tumours composed of fully differentiated plasma cells may disseminate while less differentiated tumours may be cured by excision. Pathologically the tumours form soft masses and consist of sheets of cells of uniform shape and size supported by a very delicate vascular stroma ; they bear a considerable resemblance to lymphosarcoma. Their nature can be recognized with ease if they are

composed of well-differentiated cells which stain characteristically. Recognition is difficult in poorly differentiated tumours.

Diseases of Uncertain Nature

The conditions so far described, though of unknown ætiology, are either true tumours or behave like tumours of limited invasiveness. There remain a few diseases of the reticulo-endothelial system, the very nature of which is too uncertain to permit of their being classified, though they can be grouped together on grounds of morphological similarity. In one group fall the lipoid storage diseases ; in a second, the sarcoid or tubercle-like diseases ; and the third takes the triad of Letterer-Siwe, Hand-Schüller-Christian and eosinophilic granuloma.

Lipoid Storage Diseases. Under this heading is grouped a series of complex disorders of lipoid metabolism in which there is excessive storage of an abnormal metabolite in the reticulum of the lymph nodes, spleen, liver and marrow and other tissues.

Gaucher's Disease. A chronic, congenital and familial disease beginning in childhood, lasting on the average nineteen years, but occasionally extending to thirty-six years, in which there is great splenomegaly, the average weight of the organ reaching 2,700 gm. in the adult and 1,800 gm. in the child. Accompanying this there is enlargement of the thoracic and abdominal lymph nodes and cellular infiltration of the marrow and liver. Visceral and cutaneous hæmosiderosis is present, with a typical pigmentation of the skin. There is leucopenia. The spleen, which is very firm, chocolate-brown in colour and contains infarcts and scarred areas, is studded on section with grey-white irregular masses. The liver has the same colour and contains discrete white nodules. The thoracic and abdominal lymph nodes are also pigmented and there may be nodular masses in the bone-marrow. All these tissues contain enormous numbers of reticulum cells (" Gaucher cells ") (Fig. 4/4), distended by a cerebroside. The littoral cells do not contain the lipoid but are crowded with granules of iron-containing pigment. The Gaucher cell, 20μ to 80μ in diameter in the fresh state, has an excentric small nucleus and copious cytoplasm which stains light blue with Mallory stain, looks wrinkled and contains a network of very fine threads. The spleen contains more than 10 per cent. of cerasin, which is a sphingo-galactoside.

Niemann-Pick Disease. This disease, reviewed by Baumann, Klenk and Scheidegger in 1936, usually affects female children,

who are often Jewish. Symptoms arise during the first four months of life and the average age at death is fourteen months. There is great hepato-splenomegaly and ascites, with anæmia and leucopenia. The neutral fat, fatty acid and cholesterol of the blood are raised. The average spleen weight is 209 gm. (average for the age, 30 gm.) and the liver weight 700 gm. (average for the age, 340 gm.). The spleen is doughy and soft, the liver yellow and fatty. There is generalized lymph node enlargement with yellowish discoloration. The marrow, intestinal mucous membrane, the intima of the vessels and the lungs show yellow patches and streaks. As in Gaucher's disease, the tissues contain huge numbers of reticulum cells distended by the specific lipoid. The Niemann-Pick cell (average diameter 40μ) is smaller than the Gaucher cell, the cytoplasm stains a dirty greenish-blue with Mallory's stain and is filled with fine vacuoles. The phosphatide is also taken up by cells other than those of mesenchymal reticulum, being found in cells of all descriptions, especially the alveolar phagocytes and connective tissue cells of the lung and the parenchyma of the liver. There is no hæmosiderosis as in Gaucher's disease. Chemical analysis shows the stored lipoid to be a complex mixture containing a large amount of phosphatide in the form of sphingomyelin, together with cholesterol and its esters, neutral fat being absent.

The abnormal metabolites in Gaucher's and Niemann-Pick diseases are thus closely related. Cerebroside and phosphatide are present in quite small amounts in normal tissues, but their mother substance, ceramide, is found in appreciable quantity. It thus appears that these diseases are due to a metabolic error affecting the intermediate stage of lipoid synthesis.

Sarcoidosis. Of the many names and eponyms given to this disease, sarcoidosis seems to be the most generally used and has the virtues of distinctiveness and brevity. As an entity it is difficult to define, but in the present state of our knowledge it is best to follow Ricker and Clark (1949) and Scadding (1950), and accept a histological definition. The essential lesion is a tubercle-like follicle composed of epithelioid cells surrounded by a minimal rim of lymphocytes and frequently containing giant cells. The latter are usually of Langhans' type, but may resemble foreign-body giant cells. In about 5 per cent. of cases inclusions may be found in the giant cells ; these may be the basophilic rod-like masses of Schaumann or the faintly staining asteroids of Wolbach. The follicles never show caseation, but they may

show fibrinoid necrosis. In any one site there are usually many follicles packed together and all at the same stage of development. Each is outlined by reticulin and at first contains little or no reticulin within it, but more develops as it becomes older. The follicles normally tend to heal by fibrosis and hyalinization.

Adolescents and young adults of both sexes are affected and negroes much more often than white races. The lymph nodes are chiefly involved (over 70 per cent. of cases), showing a simple painless enlargement which may decrease after a time. The hilar and mediastinal groups of glands are most frequently involved and peripheral nodes are not infrequently affected. The lungs are also often affected and show miliary shadows. In about a quarter to half of the cases the lesion appears in the deeper part of the dermis and in about the same proportion of cases the bone-marrow of the fingers and toes is involved, radiographs showing foci of rarefaction. The liver and spleen are often involved, the former probably in many cases since the diagnosis has been made with considerable success by liver puncture (Scadding and Sherlock, 1948). The other important sites affected are the eye, as an iridocyclitis, and the salivary and lachrymal glands. In all cases the disease is accompanied by relatively little constitutional upset and death from it is uncommon ; in 22 autopsied cases recorded by Ricker and Clark (1949) only 3 died as a direct result of the disease. The ætiology of sarcoidosis is unknown. A very similar histological picture can be produced by beryllium (Dutra, 1948), but this can be excluded in the vast majority of cases. Tuberculosis has naturally been suspected from the first, but tubercle bacilli cannot be demonstrated in the lesions. One difficulty in this respect is that some workers have used the absence of bacilli as a defining character of the disease. Those who have used the histological definition have found evidence of tuberculosis. Scadding records 2 cases which later had tubercle bacilli in their sputa, and Ricker and Clark were able to isolate tubercle bacilli by culture or guinea-pig inoculation in one autopsy and two biopsy cases. Another feature of sarcoid is the reaction to skin tests. The majority of cases give a negative skin test to tuberculous toxins and Lemming has found that such cases cannot be made tuberculin positive by B.C.G. vaccines, a finding that suggests a high degree of immunity to tuberculosis. At present it can only be said that the ætiology of sarcoid is unknown and that tuberculous infection cannot be excluded.

Two other conditions show a histological pattern like that of sarcoid. These are Stengel-Wolbach sclerosis and Crohn's disease. The former is an extremely ill-defined entity. The various individual cases reported have resembled sarcoidosis and many have been regarded as such by the authors. A few workers (Robb-Smith, 1938; Fowler, 1948) have suggested that the two are separate entities on histological grounds, but there does not seem to be sufficient evidence for such a distinction. Crohn's disease or regional ileitis is a condition of unknown ætiology which affects the intestine and mesenteric glands. Crohn (1949), from a personal study of 300 cases, has separated four types of the disease, chronic regional ileitis, acute ileitis, ileo-jejunitis and ileo-colitis. It is a disease of young adults of either sex and runs a chronic course with pain, diarrhœa, sometimes fever and abdominal fistulæ. At laparotomy the terminal ileum is most often affected and is reddened, œdematous and thickened (Fig. 4/5). Sometimes several isolated segments are involved. There is always great thickening of the submucosa due to lymphœdema. The muscularis may be hypertrophied and the mesenteric glands are enlarged.

Histologically the typical finding of the early stage is the presence of sarcoid-like follicles in the submucosa, subserosa and mesenteric nodes. This is accompanied by œdema, which gives place to fibrosis in the later stages, and there is always considerable non-specific cellular infiltration. Ulceration is no essential part of the lesion, but may occur as a result of secondary infection. In the later stages fibrosis and non-specific inflammatory changes dominate the picture and sarcoid-like follicles may not be found (Figs. 4/6–4/8).

The ætiology of the disease is unknown. The presence of sarcoid-like follicles suggests that it is a form of sarcoidosis, but Snapper has denied this on the grounds that regional ileitis does not occur in cases of general sarcoidosis, and Crohn also holds that the two are separate. Tubercle bacilli have not been demonstrated in the lesions. Wells and Wylie (1949), however, have noted that serum from a case of regional ileitis neutralized old tuberculin so that it would not produce a positive reaction. It is still possible that regional ileitis may prove to be of the same nature as sarcoidosis.

Non-lipoid Histiocytosis. This term has recently been used for a group of conditions which have previously been described under a variety of names and which are now believed to represent

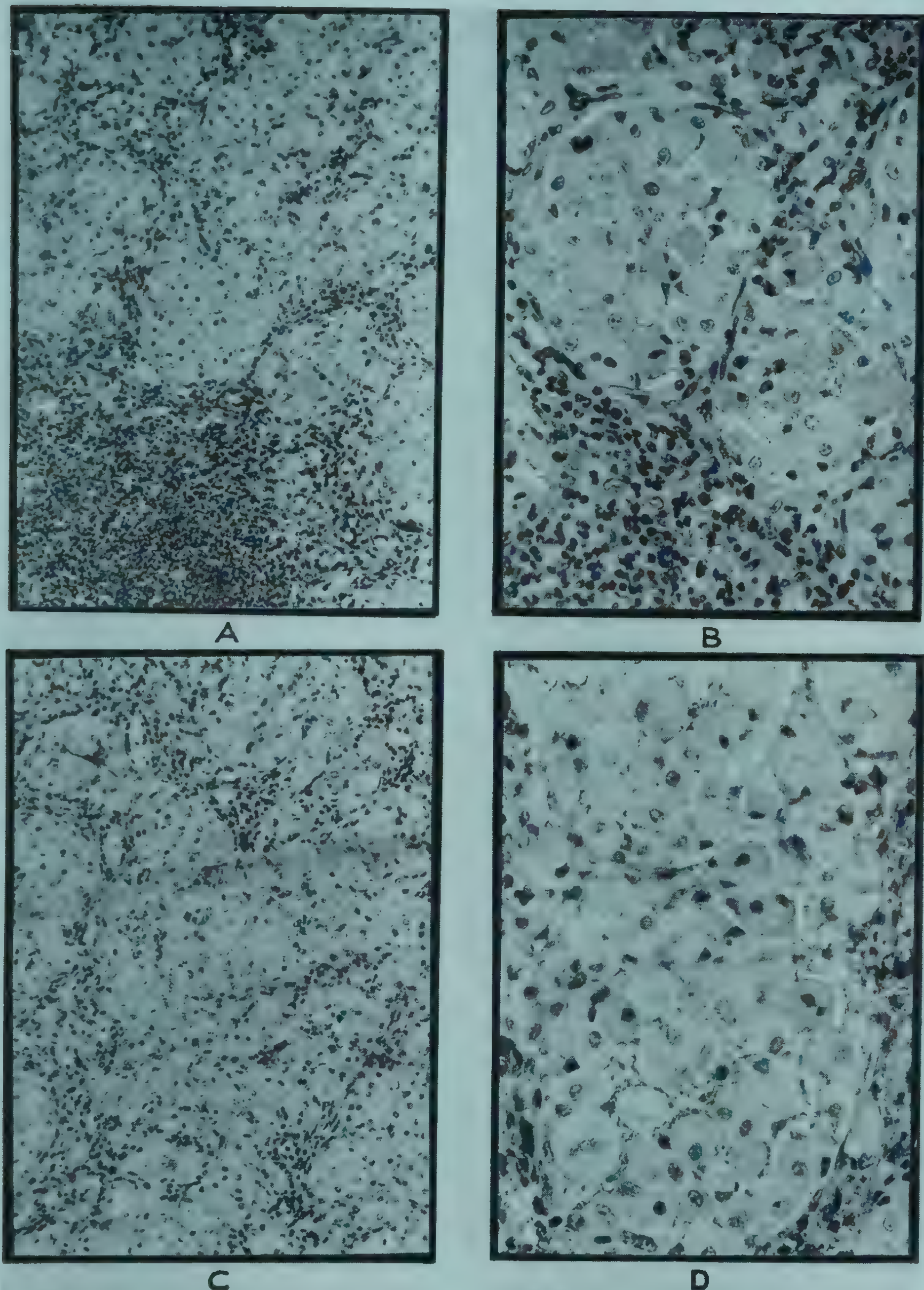

Fig. 4/4. Gaucher's Splenomegaly. A. B. Spleen : sinusoids distended by Gaucher cells. C. D. Lymph node : similar change. (*Specimens kindly lent by the late Prof. E. H. Kettle.*)

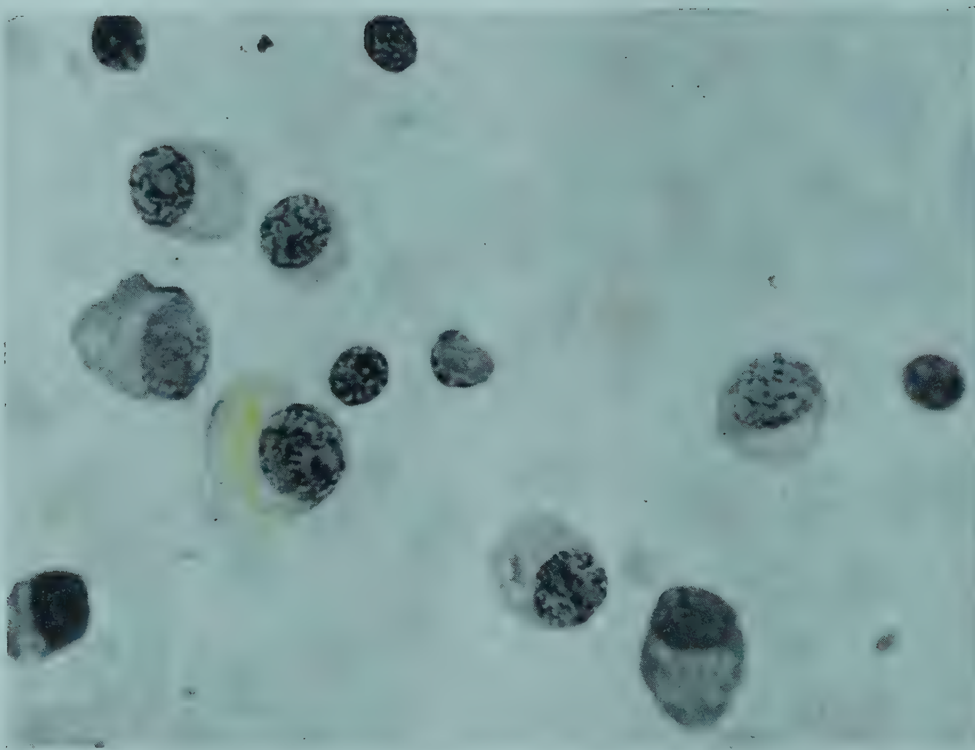

Fig. 4/3. Myelomatosis. Marrow puncture film showing numerous plasma cells. The excentric nucleus and the basophilic cytoplasm are clearly seen. The "cart-wheel" appearance of the nucleus does not appear in dried films.

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FIG. 4/5. Regional Ileitis: showing thickening and stenosis of the ileum extending to the ileo-cæcal valve. (Scale in centimetres.)

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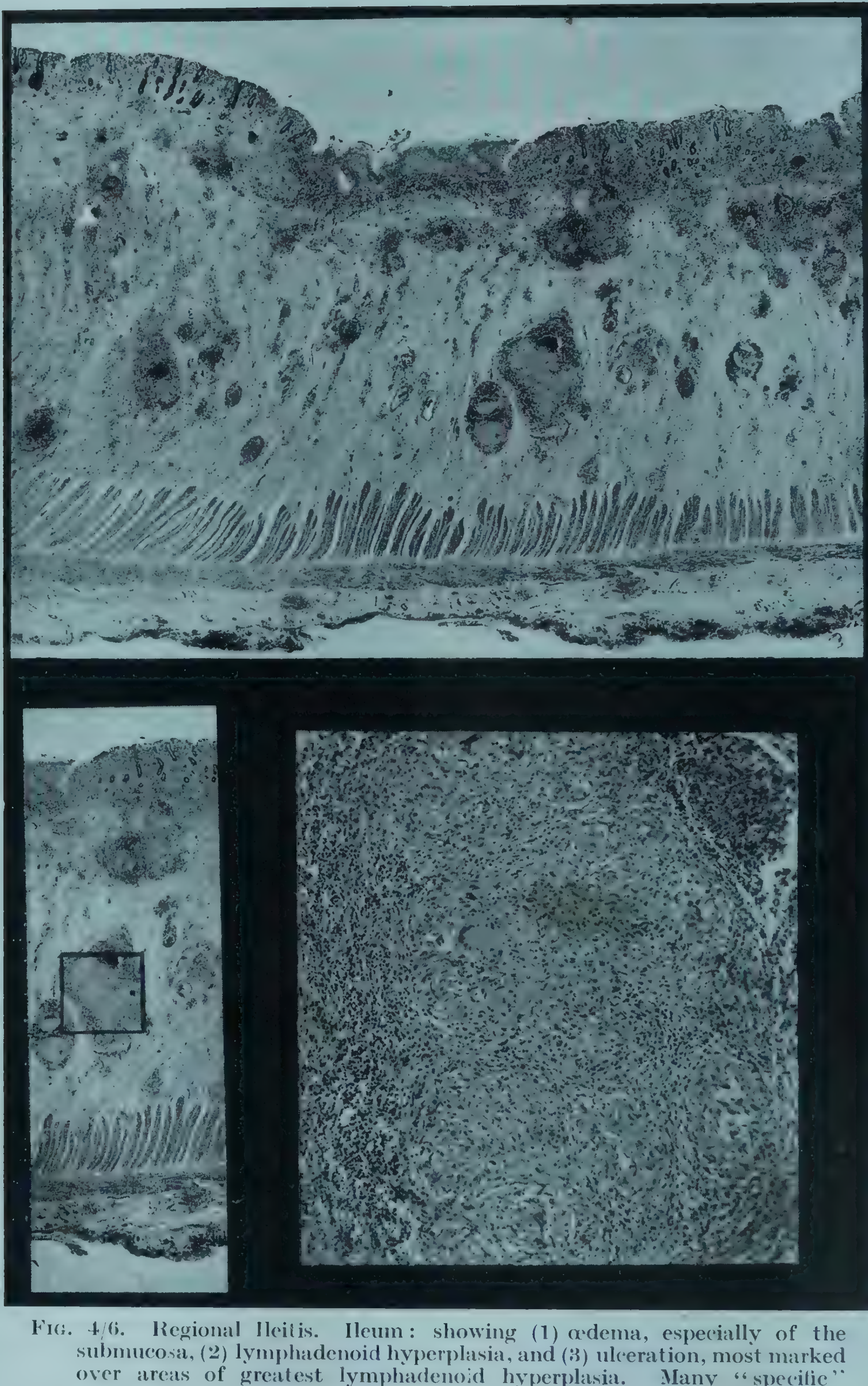

FIG. 4/6. Regional Ileitis. Ileum: showing (1) œdema, especially of the submucosa, (2) lymphadenoid hyperplasia, and (3) ulceration, most marked over areas of greatest lymphadenoid hyperplasia. Many "specific" endothelial aggregations are present. Upper × 8, lower × 8 and × 56.

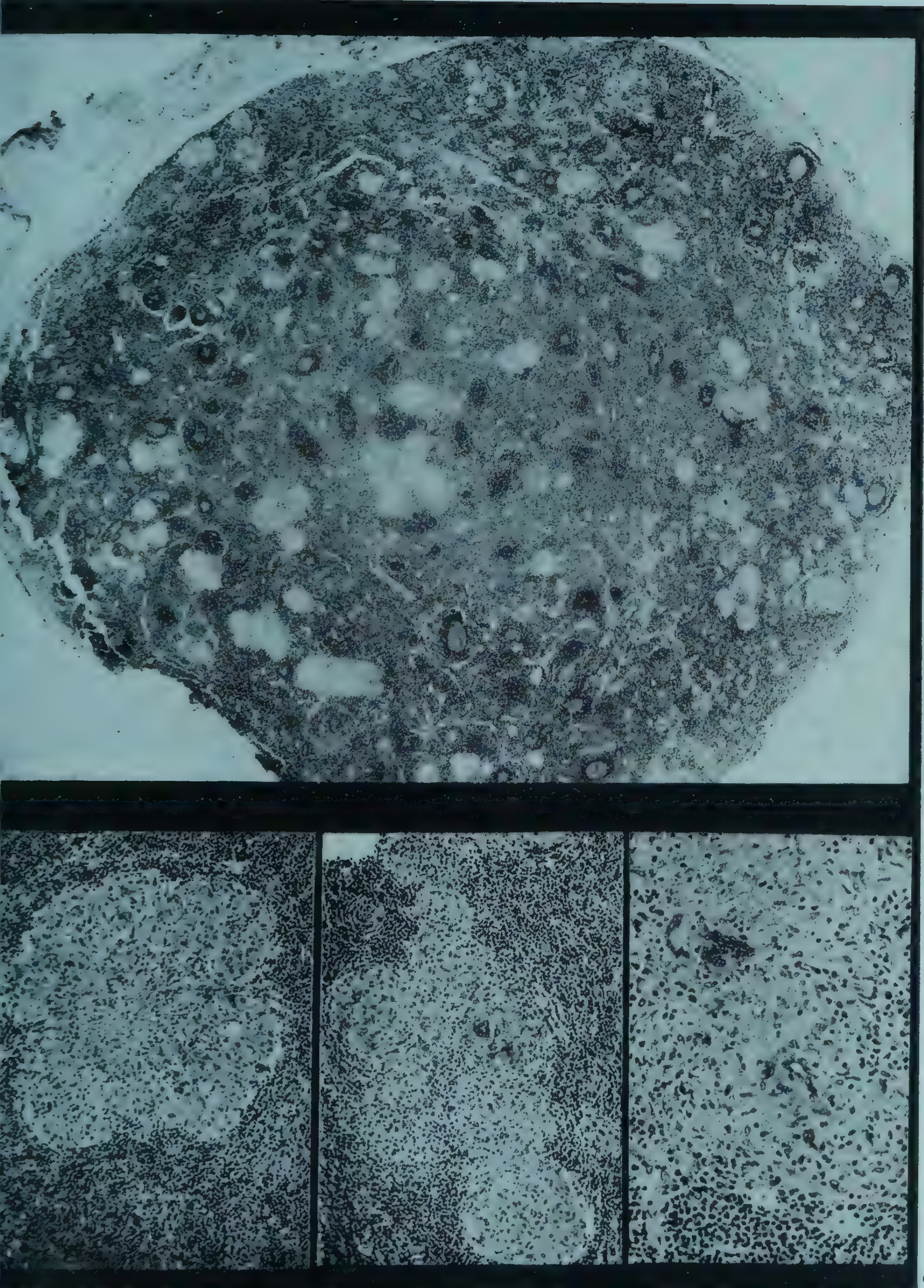

FIG. 47. Regional Ileitis. Mesenteric lymph node: showing the giant-cell systems replacing germinal centres. Upper × 10, lower × 65, × 65 and × 127.

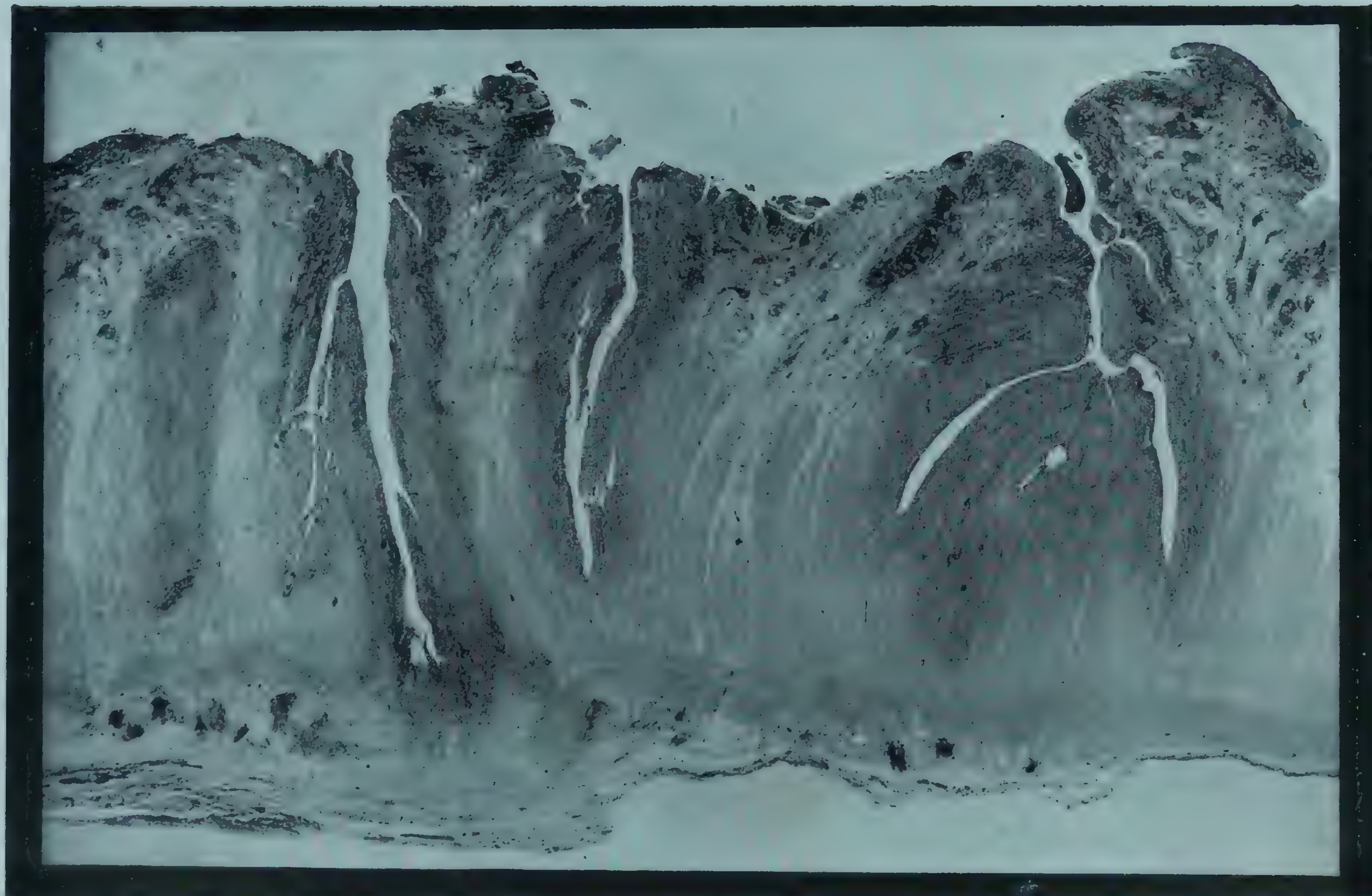

FIG. 4/8. Regional Ileitis. Ileum: showing diffuse, non-specific inflammation extending to the serosa and giving rise to many intramural fissures. × 10.

clinical varieties of one basic lesion. The three syndromes are Letterer-Siwe disease, Hand-Schüller-Christian disease and eosinophilic granuloma of bone.

Letterer-Siwe disease affects infants in the first two years of life, and runs a progressive febrile course to death in a period of weeks or months. There is usually a rash of variable type, together with ulcerative lesions of the mouth and pharynx. A moderate degree of lymphadenopathy is present, with splenomegaly and hepatomegaly, and there may be destructive lesions of any of the bones, and a progressive anæmia is usually found. At autopsy, in addition, diffuse or focal infiltration of the lungs and involvement of the intestinal lymphoid tissue are noted. Histologically the lesions are similar in all sites, characterized by an infiltration of phagocytic reticulo-endothelial cells. The infiltration, which may be diffuse or focal, does not necessarily destroy the original architecture of the tissue, and this is particularly true of spleen and lymph nodes where the normal pattern may be preserved in spite of cellular infiltration. The characteristic cells are large, variable in size, of irregular shape, and have a bulky, pale, homogeneous cytoplasm which may contain vacuoles or phagocytosed cellular remnants ; the nucleus is pale and vesicular with scanty chromatin, has no nucleolus and may be ovoid or indented. Giant cells containing several nuclei may be seen. Mitotic figures are scanty. In some cases there may be an admixture of polymorphonuclears or eosinophils, but these always form a minority. The disease is rare, but the reported cases are sufficiently numerous and resemble one another closely enough to suggest that it is a clear-cut pathological entity.

Hand-Schüller-Christian disease tends to affect slightly older children, though there is considerable overlap of age. Clinically the most striking signs are those of destructive lesions of bones, most often affecting the skull. If these lesions happen to involve the base of the skull, they may, by pressure on the eye and infundibulum, cause exophthalmos and polydipsia, and thus give rise to the classical signs of the disease, but these signs are not essential or even common. Bones outside the skull are often affected and show localized destructive lesions. Enlargement of the lymph nodes is usually seen, but splenomegaly is not common and there is rarely hepatomegaly. There may be a hypochromic anæmia. Whilst the blood cholesterol may show slight irregular elevation, it is within normal limits in most cases. The evolution of the disease is relatively slow and a considerable proportion of

cases responded well to irradiation, with reported cures after a duration of many years.

In fatal cases there are extensive destructive lesions in the skull or other bones, with soft tumour-like material replacing and spreading beyond the original bone. Lymph nodes, spleen and lungs show lesions resembling those seen in Letterer-Siwe disease.

Histologically the earliest lesions, consisting of masses of macrophages together with polymorphonuclears and eosinophils, are similar to those of Letterer-Siwe disease. In later stages cholesterol tends to accumulate, the cells become foamy and large numbers of phagocytic multinucleate giant cells appear (Fig. 4/9). In the oldest lesions the macrophages tend to break down and release this lipoid into the tissues and the whole lesion fibroses.

From this description it is apparent that the differences between the Letterer-Siwe and Hand-Schüller-Christian syndromes are slight and that there is considerable overlapping. The main differences are—the age of the child, the virulence of the process, and the prominence of bony lesions, especially in the skull.

Eosinophilic granuloma of bone affects older children, adolescents, and occasionally adults. There is usually pain and tenderness over a bone and there may be fever and an eosinophilia up to 10 per cent. Radiographs show clear-cut areas of bony destruction, usually centrally placed. The lesions occur in the pelvis, skull, ribs or proximal part of the limbs, and though symptoms are usually referable to one lesion, multiple foci have been found in some cases. The radiographic appearances are suggestive rather than diagnostic and biopsy is necessary to establish a certain diagnosis. There is no apparent involvement of any other tissue, but since the lesions are not fatal, some involvement of other reticulo-endothelial tissues cannot be excluded.

Histologically the lesions show an infiltration with large macrophages similar to those seen in the other two syndromes, but in addition there is a striking infiltration of eosinophils. According to Thannhauser (1935), the lesions pass through three

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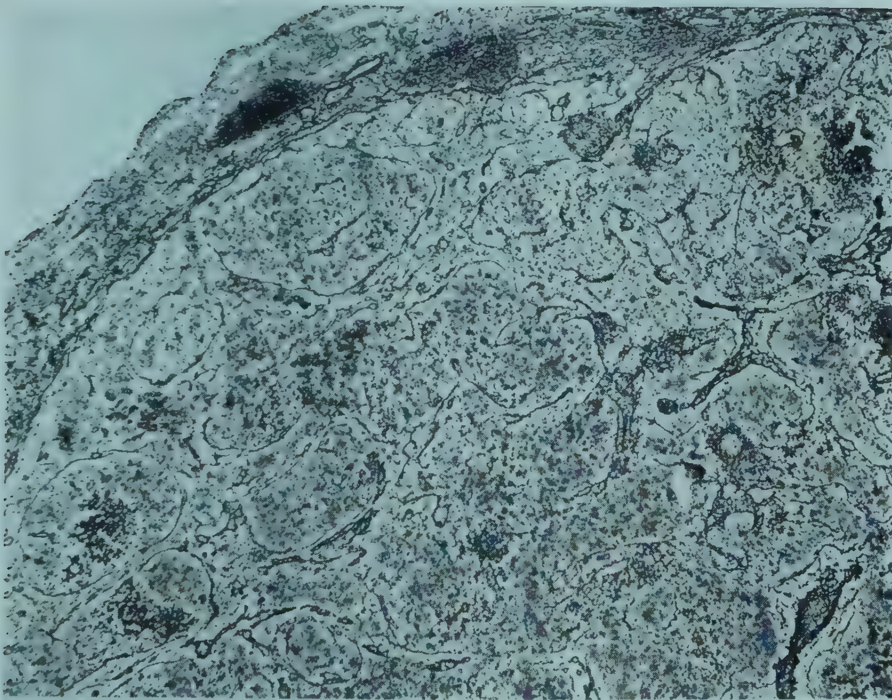
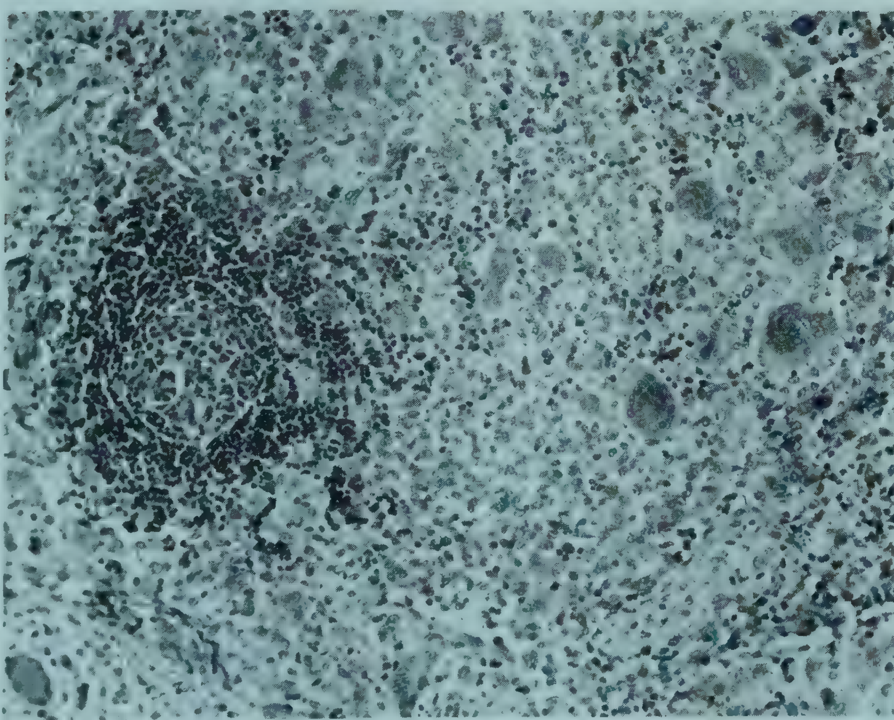
FIG. 4/9. Hand-Schüller-Christian Disease. Cervical lymph node from a boy aged four years. *Above :* Reticulin preparation showing dilatation of sinuses and loss of follicles. (× 30.) *Below :* High power of same node showing persisting small lymph follicle, replacement of normal tissue by histiocyte-like cells and presence of multinucleate giant cells. (Stained hæmatoxylin and eosin. × 145.)





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stages. The first shows the characteristic eosinophilic infiltration; this is succeeded by a proliferation of lipoid-laden foamy macrophages and giant cells, and later still by fibrosis and healing. Most of the reported cases have been permanently cured by curetting or irradiation or both, but a few have been observed to resolve spontaneously.

The relationship between eosinophilic granuloma of bone and Hand-Schüller-Christian syndrome depends on the essential histological similarity between them and also upon the occurrence of cases which clinically lie intermediate between the two diseases (Engelbreth-Holm *et al.*, 1944).

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CHAPTER V

CHEMICAL CARCINOGENESIS

Introduction

An increasing tendency to regard cancer as the end-result of a most complex procession of events has been apparent in recent years. The process begins with diverse assaults upon normal cells of chemical, physical, viral and unknown nature and ends in one essential irreversible change that is common to all malignant tumour cells, however diverse the first assaults or intervening stages. This change, whatever it consists of, is revealed as a capacity to multiply in perpetuity. A second characteristic, capacity to infiltrate, may be an expression of the first or a separate quality ; metastasis and transplantability are consequences of both. Deliberate attempts to break up the subject into separately analysable parts have become evident. Thus chemical carcinogenesis is divisible into branches which comprise the purely chemical and physical properties of carcinogenic substances, the amounts required for effective carcinogenic action, their extra- and intra-cellular location and duration of contact after application ; the exact nature of physical agents ; the problem whether the chemical or physical agents themselves or their products are effective in tumour induction ; the manner in which they react initially to give both new morphological pictures and intracellular chemical compounds or altered enzymic activities ; the remote effects in point of time and action ; mutagenic properties ; metaplasia provoking tendency ; capacity as initiators of the cancer process and promoters of tumour growth ; occurrence of natural carcinogens. Comprehensive reviews of chemical carcinogenesis will be found in the list of references (composite publication, 1947, *Brit. Med. Bull.* ; Hieger, 1949a).

Kennaway's investigations on cancer-producing substances in the 1920's[1] culminated in the discovery of pure carcinogenic chemical compounds (Kennaway, 1930), their use as a most valuable tool in the investigators' equipment and in the isolation of benzpyrene from tar (Hieger, 1930 ; Cook *et al.*, 1932, 1933).

[1] It was the writer's (I. H.) good fortune to have been Kennaway's assistant at that time ; the steps of reasoning and experiment which led to the discovery of the carcinogenic hydrocarbons are described in " Symposium on Tar and Pitch Cancer " (Hieger, 1947b).

In 1945 *half* of all experimental cancer research was concerned directly or indirectly with chemical carcinogenesis, and by 1947 over 3,000 papers had been published on this theme. Since that time the annual proportion has fallen to about 25 per cent., as judged by the gradual decline in the number of papers devoted to this branch of the subject in the principal cancer journals.

Chemical Structure and Carcinogenic Activity

With the discovery of every new carcinogen, the problem of relating activity to chemical structure becomes more difficult. These compounds now total something of the order of 300. Some of the best-known members are briefly described in Table 1. As long as attention is confined to a single group of compounds derived from one parent substance, parallelisms can be found between some chemical or physical characteristic and the carcinogenic potentiality, but such a correspondence usually fails completely when applied to another group of carcinogens derived from some other parent compound, and the same kind of difficulty is soon reached in attempts to correlate chemical structure and other biological properties.

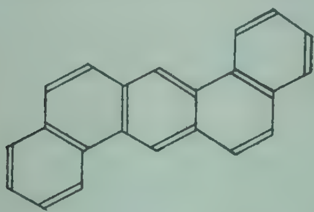
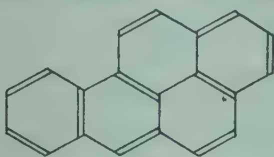
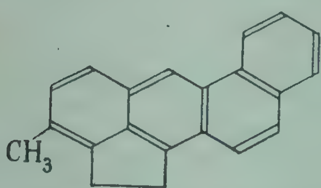
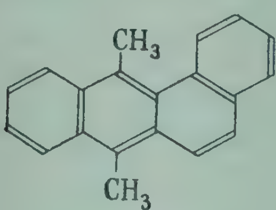
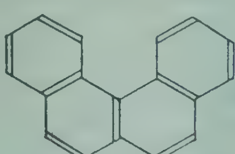
Much labour has been spent on the chemistry of the detoxicated end-products of metabolism of the carcinogens in an attempt to relate their chemical structure with the carcinogenic process (Boyland and Weigert, 1947). Pharmacologically active compounds like the carcinogens which act in small doses are much more likely to behave as catalysts than as fuel supplying energy by metabolism. The evidence which has been put forward in favour of the metabolite hypothesis rests on some facts suggesting that the metabolic end-products of carcinogenic hydrocarbons in different species of experimental animals differ *pari passu* with the variations in susceptibility to these carcinogens. It is doubtful, however, if anything is to be gained by moving the difficulties from a carcinogen on to, say, its hydroxylated metabolic elimination product, and finally, the argument that hydroxylation favours solubility is answered by the fact that hydroxyl derivatives of carcinogens are, if anything, *less* active than the parent compound.

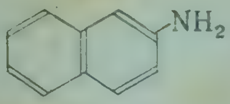
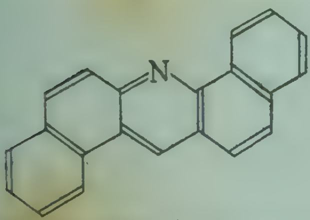
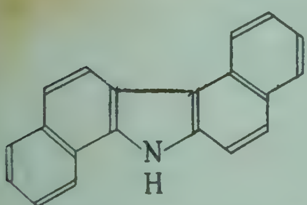
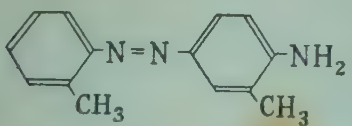
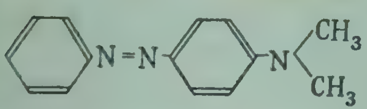
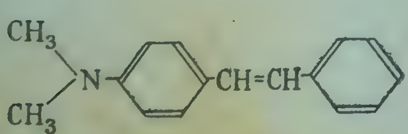
Variation in Susceptibility to Carcinogens in Different Species of Animal

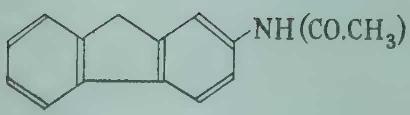
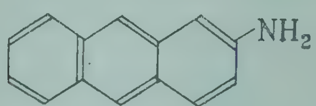
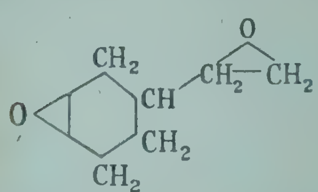
Problems of great interest are raised by striking variation in the susceptibility of different tissues and different species to the carcinogens. Even in the same species and tissue considerable

TABLE 1

Carcinogens

 1:2:5:6-dibenzanthracene	The first carcinogen (Kennaway, E. L., 1930).
 3:4-benzpyrene	Isolated from pitch (Cook *et al.*, 1933).
 20-methylcholanthrene	Has been prepared from deoxycholic acid (Cook, 1935). (This highly active carcinogen not only produces cancer of skin and sarcoma of connective tissue in the mouse, but also leukæmia and tumours of the breast when it is painted on the skin) (Kirschbaum *et al.*, 1946; Kirschbaum, 1951).
 9:10-dimethyl-1:2-benzanthracene	The most powerful carcinogen to date (Bachman *et al.*, 1938).
 3:4-benzphenanthrene	The structure of this carcinogen first showed that the 1 : 2-benzanthracene or even the anthracene nucleus was not an essential part of the molecule of a carcinogenic hydrocarbon.

 2-naphthylamine	This amine or some very closely associated compound is responsible for occupational bladder cancer in the dye industry and will induce the disease experimentally in dogs (Hueper and Wolfe, 1937).
 1:2:5:6-dibenzacridine  1:2:5:6-dibenzcarbazole	Nitrogen analogues of 1 : 2 : 5 : 6-dibenzanthracene (Lacassagne *et al.*, 1945).
 4'-amino-2:3'-azotoluene  4-dimethylaminoazobenzene (butter yellow)	Gives rise to liver tumours in the rat when given in the diet (Kinosita, 1940).
 4-dimethylaminostilbene	On injection induces subcutaneous sarcoma in the rat and mouse, also basal-celled carcinoma of the eyelid and squamous-celled carcinoma of ductus acousticus and of face, fibromata and cholangiomata of liver, mammary fibroadenomata, adenoma of lung and intestinal carcinoma and hypernephroma (Haddow and Kon, 1947).

 2-acetylamino fluorene	Incorporated in the diet produces tumours in the rat, mouse, fowl and cat. Minimal effective dose is reported to be 0·004 per cent. in diet. Tumours are of epithelial origin (1 sarcoma only in 500 rats). Has induced: glioma; squamous keratinizing carcinoma of ductus acousticus externus; carcinoma of eyelid and of retrobulbar tissue (most of these tumours originated in glandular structures); adenocarcinomata of the thyroid in mice and rats; basal-celled carcinomata of skin. Mammary cancer in rats and in mice; adenomata and metastasizing cancer of lung in rats. Tumours (benign and malignant of liver, bladder and kidney, papilloma of stomach, carcinoma of uterus; cancer of seminal vesicle in a rat (Wilson *et al.*, 1941).
 2-anthramine	Hepatoma and also basal-celled carcinomata in mice; spindle-celled sarcoma by painting on the skin of the rat (Bielschowsky, 1947).
$NH_2COOC_2H_5$ Urethane	Lung adenomata in mice by injection or by addition to the drinking water (Nettleship and Henshaw, 1943).
$N(CH_2 . CH_2 . Cl)_3$ Tri-(-2-chloroethyl)amine hydrochloride nitrogen mustard (HN_3)	Sarcoma and lung tumours in mice (Boyland and Horning, 1949).
 vinylcyclohexene dioxide	Sarcoma in rats and mice (Rose *et al.*, 1950).

variations of sensitiveness can be found as, for example, in the different strains of mice. Data on this subject have been well summarized by Andervont (1938).

Among the most resistant tissues are those of the guinea-pig,

which hardly react except to the most powerful carcinogens, and among the most easily acted upon is human bladder epithelium. In the dye-stuffs industry it has been found that a large fraction of those workmen who come in closest contact with β-naphthylamine develop bladder cancer, yet when this substance was tested in experimental animals, no positive results were obtained until dogs were used and enormous doses had been administered over a space of some years.

The resistance of the tissues of the monkey to carcinogens is an example of the puzzling variations in susceptibility of different species and tissues to carcinogenic agents. Such variations raise further difficulties in accepting the current " explanations " of carcinogenic action, because these explanations either ignore the tissues that are being acted upon, or alternatively, refer the mechanism to some chemical components which are universal to all tissue, as, for example, —SH groupings in the protein molecule.

Pfeiffer and Allen (1948) have attempted to produce cancer in monkeys with carcinogenic hydrocarbons and œstrogens. They treated fifty rhesus monkeys with œstrogen and with methylcholanthrene, dibenzanthracene and benzpyrene for periods up to ten years. Almost all organs and tissues that frequently become cancerous in human beings were treated, including the abdominal and pelvic viscera. Multiple sites were used in all animals and the mammæ were treated for the longest periods. Carcinogens were also injected intravenously and administered orally. Fibrotic and granulomatous masses and cystic encapsulation of the injected material were often produced at the injection sites and these innocent reactive changes were more pronounced with methylcholanthrene and dibenzanthracene than with benzpyrene. Pfeiffer and Allen found that :

" The lesions usually become infected and ulcerated, but if the injection site was changed, they slowly healed with typical scar formation. Sometimes there was disorientation of the tissues near the ulceration with marked papillary downgrowth of the surface epithelium. The general fibrosis consisted of large well-differentiated collagenous fibres ; the non-ulcerative tissue masses were typical granulomas with varying amounts of chronic or acute inflammation.

" Metaplasia of the mammary ducts and of the epithelium of the cervical glands occurred with both œstrogen and the carcinogenic hydrocarbons, but did not progress beyond this condition.

Despite the relatively unorganized proliferation or marked metaplasia and the formation of granulomas, a true neoplastic state was never reached. The proliferative stimulus was apparently present in appreciable amounts, but the tissue never completely lost its power of organization. All lesions gradually reverted toward the normal when treatment at the site was stopped, even though pellets of carcinogen were present in the nearby tissue."

Initiation and Promotion

The old conceptions of chronic irritation and precancerous change in tumour pathology have undergone considerable clarification in recent years as the result of experimental studies and the use of synthetic chemical carcinogenic substances. Knowledge that "irritation" is, in part at least, specific in character is derived from the use of these pure chemicals. Dissection and analysis of the cancer process has resulted in a modification of former ideas and the introduction of new terms. The new ideas and terms will first be stated, experimental evidence in support of them given later. Up to the present they apply only to induction of benign and malignant skin tumours with carcinogens of the benzanthracene series of compounds, but attempts are now in progress to extend them to other organs and compounds. Human skin is a site where the natural history of tumours may be observed. Here isolated or multiple growths arise on the basis of widespread tissue injury or stimulation which may have occurred many years previously and endured over variable periods of time. Tumours of skin of shale oil workers, cotton-spinners, those exposed to radium or X-rays, or merely to the elements of wind and sun are familiar examples. When there has been a long interval of time between the last exposure to injury or stimulus and the appearance of a growth, questions arise concerning the course of events not only from the time of the first injury, but also, in this interval, at the site of the tumours and in the intervening skin. Was the conversion to the changed neoplastic cells made all those months or years ago by some rapid reaction between particular cells and agents, and did those changed cells remain latent in the long interval, or has the process required the whole of the time to mature? In the absence of an interval between the last injury and tumour emergence is there any difference in point of numbers, in the degree of malignancy, or in the time taken for the eventual cancers to appear? Has nothing happened in the

intervening time? It cannot be said that all these long-standing questions have been answered, but a great deal of relevant preliminary information has been gained. The new ideas are due to Peyton Rous and his collaborators in the U.S.A., and to Berenblum and to Mottram in this country. Publication began almost simultaneously. The whole process, known as *tumour induction*, was studied by Rous and his associates, making use of the conditional warts of rabbit ear epidermis. Most of these growths are incapable of proliferating or even of maintaining themselves without aid, but nevertheless are true tumours of a kind having their counterparts in human pathology. Induction was divided into two phases, *initiation* and *promotion* (Friedewald and Rous, 1944). Initiation refers to that part of the process which covers the first specific assault by carcinogens on normal cells to their conversion into irreversibly changed, potentially malignant cells. Induction may next proceed uninterruptedly and imperceptibly from initiation to cell multiplication, with formation of actual visible tumours, or it may be arrested for a time or forever in the life of the animal, without revealing any further evidence of its existence. Nevertheless, *cells that have undergone initiation are capable henceforth of being stimulated by non-specific, non-carcinogenic irritants* to neoplastic multiplication. They are known as *latent potentially neoplastic cells*, though potential tumour cells might be a better term, corresponding as it does with latent tumour cells, as Ritchie (personal communication) has pointed out. The second phase, called *promotion*, refers to that part of the process which covers events from the time that the irreversible change has been made to the time when a tumour becomes visible. This is usually taken to refer to visibility to the naked eye, but logically ought to include microscopic multiplication of cells beyond what is merely hyperplastic. Promotion may be limited in time or may extend for as long as is required to keep a tumour in being. The latter may be a benign wart or malignant growth. If a wart it may regress and disappear completely in the absence of further stimulation. It is possible then for the basal cells which composed it to remain as *latent tumour cells* capable once again of being promoted into growth and of regressing again and so on repeatedly. Occasionally a rabbit wart thus treated will become malignant and maintain itself independently of any further stimulation.

It will be seen at once that the state of initiation is presumptive. Its reality can only be proved by developing or promoting it

further to the next stage of tumour growth because initiated cells have not yet been detected by visual or other means. It follows also that the agent used for promotion must itself be free of carcinogenic activity though, as will be seen later, this condition is subject to qualification. Two methods have been found of revealing that initiation has occurred. In the one, limited treatment of rabbit ears with tar or synthetic carcinogen is carried out. The treatment is planned to be insufficient by itself to induce any or more than a few tumours. The promoting agent, which does not itself induce tumours, is then brought into action after an interval in which none or a few tumours appeared. The promoting agents were trauma, chloroform, turpentine, inflammatory exudates, two of which might play a part in human pathology. If, in response to one of these promoting agents that do not themselves induce tumours, warts do in fact appear at the sites treated with both agents and nowhere else or rarely elsewhere, then this result is interpreted as proof that the carcinogen had previously initiated a neoplastic transformation which had become arrested. Finally, when the promoter was brought into action it stimulated the latent capacity for continuous cell division to the extent that a gross tumour appeared. Usually in rabbit skin the tumours are warts, but occasionally invasive growths occur. A striking example of such a sequence of events among many to be found in the papers of Rous and his collaborators, described the application of benzpyrene in benzene six times a week to one ear of thirteen rabbits during a period of twenty-seven to twenty-eight days and benzene alone to the other ear. The applications were then discontinued and four to six holes, 0·6–1·3 cm. in diameter, were punched in each ear. The healing process brought out six tumours on the ears of four rabbits treated with benzpyrene, none appearing elsewhere on the treated surfaces except a single papilloma. The ears treated with benzene only remained wholly devoid of tumours (MacKenzie and Rous quoted by Friedewald and Rous, 1950). In another experiment when ears were treated with tar, then subjected to trauma, then again tarred, tumours were more than three times as numerous, on the average, where healing had taken place and they appeared there earlier (MacKenzie and Rous, 1941). Tar itself and the synthetic carcinogens also, are all promoters as well as initiators. This was shown by alternating periods of treatments with periods without. In the free intervals no new warts appeared and those few that had arisen before disappeared. Shortly after resumption

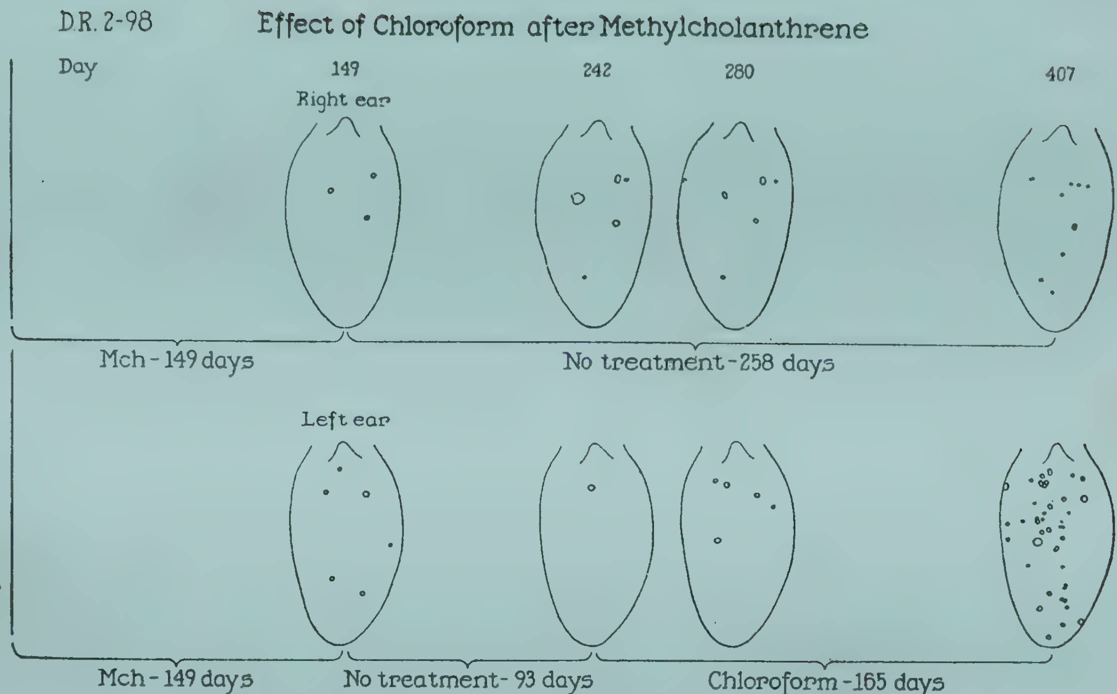


FIG. 5/1. Effect of Chloroform after Methylcholanthrene. (Friedewald, W. F. and Rous, P. 1944. *J. Exp. Med.*, **80**, p. 117, Chart 3.) *Reproduced with the authors' kind permission.*

To face page 110.

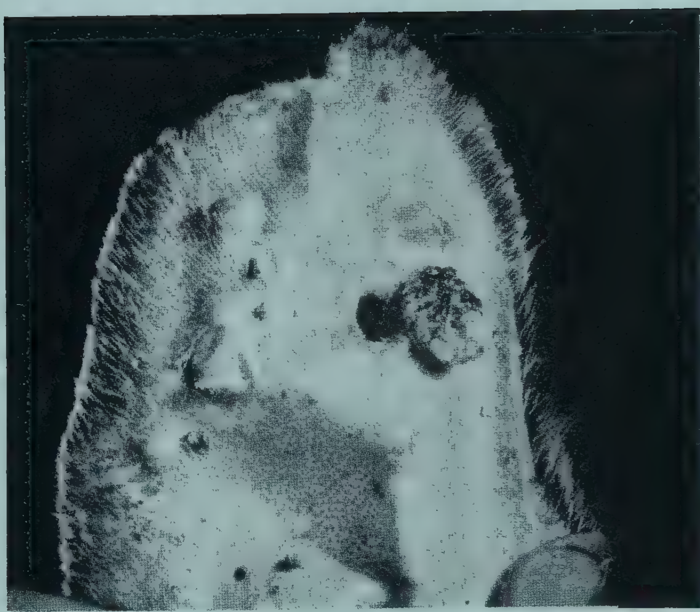


FIG. 5/2. Effect of wound healing after Methylcholanthrene. A carcinomatoid has developed at the healing edge of a hole (shown black) punched in the ear more than two and a half years after the last treatment with methylcholanthrene. (Friedewald, W. F., and Rous, P. 1950. *J. Exp. Med.*, **91**, p. 459, Fig. 13, Plate 19.) *Reproduced with the authors' kind permission.*

of tarring, some or all of those that were present before reappeared together with a large crop of new ones. Successive tarrings called forth warts in greater and greater profusion, and called them forth earlier according to these authors. The reappearance of old warts is an example of the existence of latent tumour cells, of their promotion and conditional nature. The increasing response to subsequent tarring illustrates the promoting action of tar on previously *initiated* cells. To reveal and identify this second non-specific action of tar or of synthetic carcinogens, a prompt response is of course essential. If resumption of painting with emergence of tumours were prolonged indefinitely, one could only say that the tar was acting as a carcinogen. It is because, after successive pauses, there is an increase in response and a more rapid response on each occasion that the authors believe that a carcinogenic tar applied to rabbit skin renders many more epidermal cells neoplastic than ever declare themselves by forming actual tumours. To form tumours these changed cells require a further stimulus which is supplied by trauma, carcinogenic substances themselves, chloroform, or turpentine (Figs. 5/1 and 5/2).

Search for other promoters is in progress.

The time required for initiation is unknown, nor can it be estimated until we are in possession of detailed knowledge of minimum dosage required and the time that this minimum is held in the sites affected. Judging from the observations we have in the absence of this knowledge, the time needed is widely variable. Friedewald and Rous (1950) detected warts in rabbit epidermis fifteen and seventeen days after the start of treatment with methylcholanthrene. From then on there was a continual production of tumours and presumably of tumour cells. The tumours were charted as they made their appearance and a linear increase was revealed over a period of more than five years. This increase continued after the last application of carcinogen. The authors interpret a linear increase as cellular conversion to neoplasia at evenly graded rates during the whole of this time. When they attempted to reveal the state of initiation by promotion of tumours by wound healing in the early months of treatment with methylcholanthrene, they report that few growths appeared instead of the many that should have been called forth had latent neoplastic cells been present in the multitude required to account for all the tumours arising in after years ; it would appear that in the early period relatively few cells attained to the

neoplastic state as compared with the eventual horde. These observations and the greater part of the evidence indicate that both initiation and promotion are usually lengthy processes.

In the second method of demonstrating the existence of a state of initiation, croton oil was applied to mouse skin previously treated with a dose or doses of benzpyrene inadequate to induce tumours. Berenblum (1941) found that either croton oil or resin caused tumours to appear at an earlier date than they would have arisen as a result of the treatment with carcinogen alone. Shortly after, Mottram (1944) said that a single dose of benzpyrene, if succeeded by croton oil treatment, was sufficient to induce skin tumours in mice. Although it was then known that a single application of the more powerful carcinogen, methylcholanthrene, could elicit a few skin tumours, this did not apply to benzpyrene. Berenblum and Shubik (1949b) confirmed Mottram's observation and extended it to include single applications of several other carcinogens.

The essential stages in Berenblum's technique (Berenblum and Shubik, 1947a, b ; 1949a, b) are :

1. To apply a sub-threshold dose of carcinogen to mouse skin.
2. To leave an interval (of variable length) during which the initiated cells remain dormant.
3. To apply the promoter, i.e. croton oil (originally called the co-carcinogen).

The striking fact has been revealed in these experiments that the latent period before tumours appear, after the promoter has been applied, is independent of the length of interval 2, suggesting that the initiated cells have a high degree of permanence. Berenblum has found that this rule holds even up to an interval as long as forty-three weeks (see Tables 2 and 3 taken from the publications of Berenblum and Shubik).

The constancy of the latent period is also brought out in another of Berenblum and Shubik's experiments where the concentration of carcinogen was altered. The variation in the number of skin tumours per mouse suggests the existence of cells or cell groups of varying degrees of susceptibility in the skin, i.e. the low concentration of carcinogen can initiate only the most sensitive centres, whereas the stronger solution can act on the less sensitive centres as well.

The latent period (measured from the beginning of the croton oil treatment) is independent of the carcinogen as is shown in the table, which is the more remarkable because dimethyl-

benzanthracene is generally considered a much more potent carcinogen than dibenzanthracene.

TABLE 2

The Influence of Interval between the Single Application of Carcinogen (9 : 10 *dimethyl*-1 : 2-*benzanthracene* (*DMBA*)) *and the Croton Oil Treatment* (*Twice Weekly for Twenty Weeks*)

Series	Interval	No. of Mice Used	Survivors at Time of First Tumour	Mice with Tumours	Percentage with Tumours	Average Latent Period in Weeks	
						(*a*)	(*b*)
C	3 days	92	62	36	58	9·8	9·5
D	5 weeks	92	80	46	57·5	11·2	6·2
E	10 ,,	95	48	35	73	16·8	6·8
F	15 ,,	45	24	18	75	23·6	8·6
G	20 ,,	46	26	15	57·5	25·5	5·5

(*a*) Latent period counted from commencement of the experiment.
(*b*) Latent period (*a*) with interval deducted.

TABLE 3

Series	Treatment [1]	No. of Mice Used	Survivors at Time of First Tumour	Mice with Tumours	Percentage with Tumours	Average Latent Period (Weeks)
A	BP and croton oil .	45	40	15	37·5	10·6
B	DBA and croton oil.	64	37	11	29·5	10·1
C	DMBA and croton oil	92	62	36	58	9·5

[1] Treatment consisted of one application of the carcinogen, followed by twice weekly applications of croton oil for twenty weeks.
Carcinogen : BP —0·8 per cent. 3 : 4-benzpyrene in liquid paraffin.
DBA —0·3 per cent. 1 : 2 : 5 : 6-dibenzanthracene in benzene.
DMBA—1·5 per cent. 9 : 10-dimethyl-1 : 2-benzanthracene in liquid paraffin.

Berenblum and Shubik conclude that dibenzanthracene is as potent an initiator as dimethylbenzanthracene, but that its more feeble activity lies in its less powerful action as a promoter. Promotion in this experiment is due to croton oil.

Berenblum's earlier investigations on co-carcinogens led him to postulate a three-stage process for carcinogenesis, but his more recent work has shown that a two-stage process (initiation and promotion) would describe the changes more adequately. He states that :

1. When carcinogens are repeatedly applied to mouse skin, the proportion of cancerous mice tends to rise to 100 per cent. ; that is to say, that given long enough, *all* the mice will develop tumours.

2. On the other hand, if a minimal treatment with carcinogens is given (i.e. one painting only), and is followed by prolonged painting with croton oil, then a certain proportion *only* of the mice develop tumours, however long the painting with croton oil is continued.

3. Furthermore, if there is an interval of any length of time up to twenty weeks between the single treatment with carcinogen and the beginning of painting with croton oil, tumours develop after a time *independent* of the interval between carcinogen and croton oil painting.

Berenblum states that these facts " could be explained by considering the preliminary carcinogenic action as causing an irreversible conversion of a few normal cells into a few latent tumour cells, and by assuming that the croton oil converts these latent tumour cells into visible tumours."

Berenblum, applying the initiation-promotion nomenclature to the characteristic property of the carcinogens, says :

" Another permissible conclusion is in regard to the anomaly that has always existed in assessing carcinogenic potencies. It is known from the reliable data of Bryan and Shimkin (1943) concerning the potencies of 1 : 2 : 5 : 6-dibenzanthracene, 20-methylcholanthrene and 3 : 4-benzpyrene, for the subcutaneous tissues of the mouse, that the order of carcinogenicity is DBA : MC : BP when judged on the basis of minimal dose-response, but that it is MC : BP : DBA when judged on the basis of average latent period. It appears from the present results, that there is no real anomaly, since the minimal dose-response is a measure of initiating action, while the average latent period is a measure of promoting action. Dibenzanthracene is undoubtedly a potent initiator, but a weak promoter ; benzpyrene is moderately potent, both as initiator and promoter ; croton oil, on the other hand, is exceptionally potent as a promoter, but quite useless as an initiator."

It may be said that these conceptions do not differ materially from our former ideas about pre-cancer. In one respect there is no difference. No precise morphological description can be given either of cells that used to be called " precancerous " or of cells that are now said to be " initiated." Nevertheless, the state of initiation is definite and unique. After it has been brought about,

cell function is changed and with it the response to certain non-carcinogenic irritant stimuli. The term " irritant " is here used in a general sense as something which by itself either directly or indirectly calls forth a response other than necrosis in a normal cell. The response to an irritant acting upon an " initiated " cell consists of progressive and continuous multiplication as revealed by warts and malignant growths. It seems reasonable to equate the action of these non-specific irritants with " chronic irritation " of human pathology. We may guess, for instance, that a blue-black mole consists of initiated cells readily promoted by trauma. The widespread existence of cells undergoing initiation, and of others which are latent or potentially neoplastic, supports a belief in the regional rather than local origin of tumours (Willis, 1948). Individual growths may seem to be unique. This is so only because they are the first to be promoted among the many latent or gradually evolving neoplastic cells within a region.

Quantitative Relations in Chemical Carcinogenesis

Dose required for Tumour Production. Several investigators have succeeded in inducing skin papillomas in mouse skin by a single application of carcinogen (e.g. methylcholanthrene); if it be assumed that a single drop was applied, this dose would be of the order of 0·2 mgm. The application of 0·003 per cent. 1 : 2 : 5 : 6-dibenzanthracene biweekly to the skin of mice for about a year has also given positive results, and here the dose was of the order of half of that in the single application experiment.

It is by no means easy to arrive at a true figure for the minimal dose required, for the carcinogen is distributed among all the cells at the site of application and the actual number of cells converted to tumour cells is still unknown, although the evidence brought forward on this point favours the cancerization of a considerable number of cells and not of a minute locus consisting of a few or even of one specially sensitive cell (see, for example, the view put forward by Willis, p. 152). Such considerations might suggest the speculation that the minimum dose required for tumour production is equivalent to the minimum number of cancer cells which will develop into a tumour.

One of the most practical methods of investigating the quantitative aspect is by examining the " die away " region of the graph relating dose with tumour response. The papers of Bryan and Shimkin (1941, 1943) give numerous tables dealing with this

relation. The figure in Table 6, showing a tumour induced with 0·00195 mgm. benzpyrene, is perhaps in some doubt, because it has been shown that cholesterol and some of the other lipoids used as solvents for the carcinogens are themselves weakly carcinogenic.

TABLE 4

Tumour Production by 1 : 2 : 5 : 6-*dibenzanthracene* (*Subcutaneous Injection*)

(Summary of data of Dobrovolskaia-Zavadskaia)

Dose in Milligrams	Mice Injected	Mice with Tumours	Tumour Incidence
			Per cent.
0·01	328	37	11·28
0·005	364	13	3·57
0·0025	167	2	1·20
0·00125	158	0	0

TABLE 5

Tumour Production by 1 : 2 : 5 : 6-*dibenzanthracene* (*Subcutaneous Injection*)

(Summary of data of Lettinga)

Dose in Milligrams	Mice Injected (Original Total)	Mice with Tumours	Tumour Incidence
			Per cent.
5·0	10	5	69·25
2·5	10	8	100·0
1·0	10	9	98·0
0·5	10	10	100·0
0·25	30	26	90·2
0·125	20	9	53·8
0·05	10	4	45·3
0·0125	20	4	32·3
0·005	20	0	0

The tumour incidence percentage is calculated on the number of mice which have survived the minimum latent period.

TABLE 6

Incidence and Latent Period of Tumours in Mice Injected with Benzpyrene

Dose in Milligrams	Mice Injected	Mice with Tumours	Mean Latent Period	Tumour Incidence
			Months	Per cent.
8·0	21	20	2·96	99·5
4·0	19	16	3·02	100·0
2·0	19	19	3·09	100·0
1·0	20	18	3·32	94·7
0·5	19	19	3·86	100·0
0·25	21	14	4·41	66·7
0·125	19	15	5·11	83·3
0·625	20	4	5·79	20·2
0·31	16	0		0
0·156	19	0		0
0·0078	40	0		0
0·00195	81	2	8·37	2·97

The tumour incidence percentage is calculated on the number of mice which have survived the minimum latent period.

TABLE 7

Incidence and Latent Period of Tumours in Mice Injected with Methylcholanthrene

Dose in Milligrams	Mice Injected	Mice with Tumours	Mean Latent Period	Tumour Incidence
			Months	Per cent.
1·0	20	20	2·40	100
0·5	21	21	2·57	100
0·25	21	21	2·77	100
0·125	21	21	3·32	100
0·062	21	17	3·92	86·3
0·031	20	13	5·24	65·3
0·0156	18	6	4·59	36·4
0·0078	17	3	6·97	19·0
0·0039	19	0		0
0·00195	19	0		0
0·00098	41	0		0
0·00024	79	0		0

The tumour incidence percentage is calculated on the number of mice which have survived the minimum latent period.

TABLE 8

Incidence and Latent Period of Tumours in Mice Injected with Dibenzanthracene

Dose in Milligrams	Mice Injected	Mice with Tumours	Mean Latent Period	Tumour Incidence
			Months	Per cent.
8·0	21	16	3·75	100
4·0	20	17	3·83	91·5
2·0	19	19	3·69	100
1·0	22	22	3·60	100
0·5	21	20	3·76	99·5
0·25	21	19	4·01	95·0
0·125	23	21	4·47	95·9
0·062	20	20	5·10	100
0·031	21	16	6·31	80·8
0·0156	19	6	5·94	38·0
0·0078	40	6	8·78	18·7
0·00195	79	2	9·48	3·2

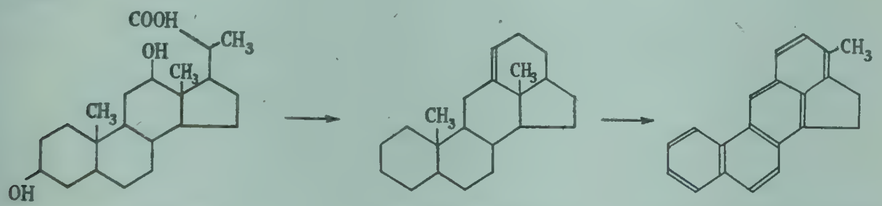
The tumour incidence percentage is calculated on the number of mice which have survived the minimum latent period.

Relation of Structure to Potency of Carcinogens. One of the main problems awaiting solution in the structure-potency relation is that comparatively minor changes in the structure of the carcinogen can have a profound influence on their activity. These facts are illustrated by tables in the paper entitled " Symposium—Industrial Skin Cancer, Chemical Aspects of Industrial Skin Cancer caused by Pitch and Tar " (Hieger, 1947b), although it should be remembered that observed differences of activity are partly due to factors depending on " variations of susceptibility " of the experimental mice. The changes in potency effected by major changes of structure are less surprising. These " variations of susceptibility " constitute one of the most attractive, elusive and difficult themes in cancer research.

Carcinogens of Biological Origin

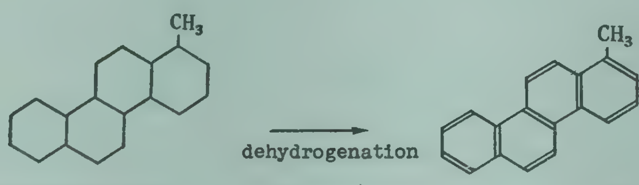
The rapid increase in the length of the catalogue of carcinogens has had some awkward consequences. The sex hormones were discovered at about the same time as the carcinogens, and since the structural nucleus of the hormones and of the earlier carcinogens showed resemblances, the startling idea was then put forward that human cancer might be caused by endogenous

carcinogens originating in some error in synthesis or metabolism of the sex hormones. This possibility was extended to the bile acids when Cook (1935), showed, by preparing methylcholanthrene from deoxycholic acid, that a powerful carcinogen could be prepared directly from a steroid, although by somewhat drastic means. Further, the rule that substitution of the 1 : 2-benzanthracene molecule in the 5, 9 or 10 positions conferred carcinogenic activity still held when the substituents formed a bridge.



Deoxycholic acid dehydronorcholene 20-methylcholanthrene

With the discovery of carcinogens which were not hydrocarbons or did not possess structures of fused ring steroid type, e.g. stilbenes, azo dyes, acetylaminofluorene and urethane, the idea of carcinogens being created as the result of faulty metabolism lost favour. Nevertheless, Shoppee in 1947 proposed that since methyl chrysenes had been shown to be carcinogenic, steroids of the adrenal cortex, from which the methyl chrysenes could (on paper) be derived, might be implicated as a source of carcinogen, e.g. :



(17a-methyl-D-homoandrostane) (methyl-chrysene)

Among the latest carcinogens at the time of writing (1951) are members of the nitrogen mustard and epoxide class. The former would present some formidable difficulties in any theoretical derivation from a tissue constituent, but the hypothetical formation (on paper at least) of one of the simplest carcinogens, urethane, would present no serious obstacles.

The most recent results in the search for naturally-occurring carcinogens, first discovered by Shabad, show that :

1. A variety of animal and plant fats are weakly carcinogenic, especially if they have previously been heated to 300° C.

2. The unsaponifiable fraction (i.e. chiefly cholesterol and associated compounds) of the fat of human and animal tissues is carcinogenic, and although the potency is much lower than that of the classical carcinogens (1 : 2 : 5 : 6-dibenzanthracene, benzpyrene and methylcholanthrene), yet it is distinctly higher than that of the fats before saponification.

3. The cholesterol-rich fractions from the fats of the tissues of human subjects who have died of cancer is not more carcinogenic than fractions similarly prepared from the tissues of subjects who have died of other diseases.

4. Commercial cholesterol (made *via* the saponification of brain and spinal cord of cattle) is no less and often more carcinogenic than the cholesterol fraction from human tissues.

The carcinogenic activity of some of these lipoid substances is shown in the table :

TABLE 9

Showing Characteristics of some Carcinogenic Substances of Biological Origin

Source (C = from human subjects who died of cancer. Non-C = from human subjects who died of other causes)	*Material*	*Carcinogenic Assay* (No. of sarcomas induced at site of subcutaneous injection/total number mice treated)	*Author*
C . . .	Benzol extract of liver.	4/179	Kleinenberg, Neufach and Shabad (1940).
Non-C . . .	,,	0/91	
C . . .	Unsaponifiable fraction of liver.	12/56 12/456 17/101	Steiner (1942–43). Steiner *et al.* (1947).
Non-C . .	,,	5/63 10/440 44/120	
C . . .	Benzene - ether extract of 12 livers.	0/131	
C . . .	Unsaponifiable fractions of 4 human cancers	0/45	
Sesame oil . .		0/18	
Sesame oil after heating to 350°.		3/31	
Non-C . .	Unsaponifiable fraction of livers of still-born infants.	3/39	
Unsaponifiable fraction of pig's liver.		4/115	
Unsaponifiable fraction of beef liver.		0/115	

Source (C = from human subjects who died of cancer. Non-C = from human subjects who died of other causes)	*Material*	*Carcinogenic Assay* (No. of sarcomas induced at site of subcutaneous injection/ total number mice treated	*Author*
C	Unsaponifiable fraction of liver.	11/28	Hieger (1940, 1946, 1947*a*, 1949*b*).
C and non-C .	Unsaponifiable fraction of Bantu livers.	5/30	
C	Cholesterol-rich fraction of liver.	2/10	
Non-C . . .	,,	0/20	
Non-C . . .	Cholesterol-rich fraction of liver, lung, kidney and muscle.	2/25	
C	,,	3/25	
Cholesterol rich fraction of cream of cow's milk.		2/20	
Commercial cholesterol (from brain and spinal cord of cattle).		25/436	
Lard		5/50 (rats)	Burrows, Hieger, and Kennaway (1932, 1936).
C	Fatty extract of human breast cancer ; four solvents.	7/36	Menke (1942).
Non-C . . .	Fatty extract of human breast; four solvents.	0/54	
Benzol extract of rat fibrosarcoma .		5/28 (rats)	Aptekman *et al.* (1943).
Benzol extract of rat liver . .		2/24 (rats)	
Wheat germ oil		10 per cent. of rats injected with the oil developed sarcoma.	Harris (1941).
Cotton seed oil heated to 350° for one hour.		2 sarcomas in 12 mice.	Beck (1941).
Cotton seed oil heated to 210° for twelve hours.		0 sarcomas in 12 mice.	
Cholesterol heated to 270°–300° .		2 sarcomas in 30 mice.	Beck *et al.* (1945).
Cholesterol esters heated to 300° .		0 sarcomas in 40 mice.	

The facts set out in the table have had an odd reception from some quarters, as though the term "carcinogen" should be limited to potent carcinogens which can evoke cancer in 100 per cent. of animals in a few months, and should not be applied to agents whose activity in this respect is less spectacular, and as though the isolation of carcinogenic substances from the tissues of non-cancerous animals (human or otherwise) were at least a disappointing result if not a demonstration that this line of attack on the cancer problem is an error. Another objection expresses itself as a reluctance to accept the idea that cholesterol is a carcinogen and to prefer to transfer the potency of commercial cholesterol to the impurities associated with the pure sterol. This second objection is based on the inference that since cholesterol is present in all tissues, it therefore cannot be a carcinogen, for if it were, all tissues would finally become cancerous. However, it might be pointed out that the body contains other substances (e.g. HCl), which at the appropriate place and concentration are essential physiological agents, yet in abnormal amount or location would be lethal.

The catalogue of carcinogens has indeed become embarrassingly long, and the announcement of the carcinogenicity of more or less familiar chemical compounds should no longer cause great surprise; in the past the carcinogenic capacity of a group of compounds has sometimes been unsuspected, due to the undeveloped state of the technique for testing these particular substances. Who would have guessed that urethane and chloroform (Eschenbrenner, 1945) were carcinogens ?

The potency of commercial cholesterol is by no means negligible, as the table shows, and if the calculation is made so as to include as experimental animals at risk only those which survive to the average tumour age, the incidence rises to about 20 per cent., which is of the order of incidence of cancer in human subjects. Furthermore, the fats and cholesterol-rich fractions of tissue are very slow-acting carcinogens, i.e. tumours occur in the senescence of the experimental animals. This feature of the biological carcinogens renders them less attractive for use by experimental cancer investigators, who naturally prefer speedy results. Nevertheless, the order of potencies and the prolonged latent period of carcinogenesis by substances derived from biological materials suggests a closer analogy with the process of spontaneous cancer than do the powerful synthetic carcinogens, although any hypothesis of human cancer implied by such an analogy at present rests on specially selected facts.

For example, Leary (1950), who approached the problem from precisely the opposite direction, states in a summary : " Evidence is presented that the crystalline ester cholesterol deposited focally in the epithelium of renal convoluted tubules in adults, particularly in those afflicted with nephrosclerosis, is the stimulating agent responsible for the growth of benign cortical adenomas in man. Removal of the crystalline ester cholesterol is followed by a cessation of the growth of such adenomas, which then revert to inactive tubular adenomas. It is generally accepted that adult renal cortical adenocarcinomas have their origin in benign adenomas."

One difficulty in the way of accepting the carcinogenicity of lipoids *per se* is the question of possible contamination of lipoid by air-borne benzpyrene in soot. Fortunately, this problem is by no means insoluble.

To resume, when the aromatic polycyclic hydrocarbon carcinogens, and particularly methylcholanthrene, were brought to the aid of cancer research, the hope was raised that their chemical similarity to the structural skeleton of such molecules as cholesterol, bile acids, vitamin D and the sex hormones, would supply the key for the solution of the problem of " spontaneous " cancer. Although no carcinogen of the hydrocarbon type has yet been detected in human tissue, several plausible explanations might be advanced in defence of the hypothesis ; for example, that the carcinogen represents a fleeting moment in the rapid series of chemical changes that go on in tissue, and that as soon as it is formed, it is detoxicated in preparation for elimination or for further metabolism. Proponents of the chemical hypothesis of human cancer might perhaps draw a little consolation from the facts described above, namely, that the lipoids, which occur in all tissues, can under appropriate conditions behave as slow-acting carcinogens.

Cancer in Industry and Risks associated with the Industrial Application of Atomic Energy

New materials are constantly being introduced into industry, and it would be surprising if some of these were not potential cancer producers. The long latent period before malignant changes are evoked in industrial cancer can only too easily conceal the dangers to which the workers are being exposed. For example, the use of beryllium salts as a component of the fluorescent layer in lighting tubes entails certain risks, for this metal is highly toxic, causing fibrosis and granulomas when accidentally embedded

in the skin (if a tube is broken) or by inhalation of the dust. Beryllium salt mixtures, when experimentally injected intravenously into the rabbit, give rise to osteosarcomas of tibia and scapula with metastases in lung and liver ; in one experiment 6 out of 9 rabbits developed these tumours after one year (Dutra and Largent, 1950 ; Dutra, 1949).

The petroleum industry has for many years been using substances like Al_2O_3 on a large scale in the catalytic cracking process. These catalysts acting on petroleum residues, yield by disintegration and re-synthesis simultaneously, new high and low boiling products, some of which have been used as pitch and resin substitutes, for instance, as rubber plasticizers. It may be recalled (Hieger, 1947b) that Kennaway used a very similar method (namely, $AlCl_3$ acting on tetrahydronaphthalene at moderate temperatures) for the synthesis of carcinogenic preparations containing 3 : 4-benzpyrene.

The by-products obtained by an extraction process and also catalytically from petroleum have proved to be strongly carcinogenic. The admixture of such carcinogenic substances in rubber articles or other materials of everyday use can only be described as inviting skin cancer. However, it would be difficult to discover to what extent these dangerous products of the petroleum industry have indeed been incorporated into materials in common use ; certainly during the late war, government authorities regarded the matter with concern, and one large petroleum company has given wide publicity to the measures essential for safeguarding their workpeople from catalytically prepared oils. The use of compounds which induce cancer at sites remote from the point of administration, e.g. urethane, adds a further potential cause of industrial cancer. How far the use of these toxic agents is unavoidable, and to what degree protection could be achieved by thoroughgoing mechanization is a matter for serious thought. One can hardly fail to be reminded here of some of the abhorrent practices of the past, such as the employment of naked boys for cleaning chimneys, or the use of carcinogenic shale oil for lubricating cotton-spinning machinery, of β-naphthylamine in the dye industry or of butter-yellow (p-dimethylaminoazo-benzene) for colouring foodstuffs. Alternatives must be found for these dangerous products and processes. It is to be hoped that β-naphthylamine, after many years of use (or misuse), will shortly be abandoned as an intermediate in the dye industry in this country.

Obviously the new radio-chemical industry and research has led to new risks and dangers. However, since this kind of work is now under state control and has been started at a time when the dangers are well known, careful attention is being paid to the risks of inhalation of radioactive dusts, and to the possibility of the dissemination of such materials into the atmosphere or as effluent into rivers.

Cancer has been experimentally induced in mice by some of the radio-elements which have become so prominent in the last few years (e.g. plutonium). It has been found (Lisco *et al.*, 1947) that the injection of 1μ g. of Pu^{239} into mice will bring about greying of hair, epilation, ulceration of the skin, destruction and atrophy of the muscles and local malignant fibrosarcomas in not less than 200 days. Similar changes result from the injection of radio-yttrium and radio-cerium 91, and of another fission product from the atomic pile, namely, strontium 89, which rapidly settles down in bone. Tumours have thus been experimentally induced in mice, rabbits and rats in bone, e.g. in the spine, long bones, pelvic bone, jaws and ribs. According to Peller (1939), the workers at Joachimstal (where the uranium ores were mined for the Curies' momentous discovery) had in the past, at least, a very high cancer death rate, chiefly of the lung, and amounting to 53 per cent. of deaths from all causes ; in one series of statistics 42 out of 47 of the miners' cancers were thoracic.

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CHAPTER VI

EXPERIMENTAL CANCER RESEARCH

VIRUS HYPOTHESIS AND SARCOMATOUS TRANSFORMATION

ONE of the difficulties of accepting the idea that cancer may be caused by one or a multitude of viruses relates to nomenclature. The designation " virus " is regarded by some as too precise for the purpose, by others as itself too ill-defined to be of value in our present state of ignorance. The term originally used by Peyton Rous in relation to the chicken tumours (1911) was *tumour agent.* Later by common consent, virus was adopted owing to certain properties possessed in common by these infective, formative entities which multiply intracellularly, and the previously known infective cytoclastic viruses. The properties concerned were, invisibility in ordinary white light with the microscopes then in use ; filterability ; capacity to multiply within infected cells ; to withstand X-radiation but not ultra-violet light ; to survive freezing and drying and to provoke antibodies on injection into other animals. At the time that " virus " was substituted for " tumour agent," no tumour virus nor any cytoclastic virus had been characterized precisely in terms of chemistry and physics. Some of the cytoclastic viruses have since been so defined, but because tumour viruses have thus far eluded purification and accurate description, pedantry proscribes the use of the term virus. Nevertheless, it will be used now as formerly on account of the common biological characters referred to.

Rôle and Properties of Tumour-inducing Viruses

Any attempt to explain why a cell differs from normal in being neoplastic has to account for its capacity for continuous multiplication in the apparent absence of any stimulus. The need for a stimulus is assumed. This capacity for continuous multiplication is seen in primary malignant tumours and in their metastases. That malignant tumours may even grow in perpetuity has been made manifest by continuous propagation from host to host of tumours that first arose early in the century and are now proliferating as vigorously as ever. There is no sign that they will

ever stop, so long as suitable hosts are provided. The implication of these facts is that the cause of this continuous multiplication is carried along with, or in, the affected cells. The continuing cause or stimulus cannot be the non-living chemical substance or physical agent or its products which originally caused the tumour. These will have been eliminated by dilution in the course of time, unless the irreversibly changed cells have become capable of synthesizing them or their effective products. No such synthesis has yet been demonstrated. If, however, the initiating chemical substance is a living one, capable of reproduction in the host's cells and of transmission from one somatic cell generation to another, then a means exists of perpetuating both the cause, the tumour virus, and the response to it revealed as cell multiplication. From this point of view, a living agent meets the requirements both for a stimulus and for its inheritance by somatic cells. It infects, and for some reason as yet unknown, stimulates cell division. Since the virus or agent itself multiplies in the host's cells, it will be carried from one cell generation to the next, either with nuclear chromatin or with cell cytoplasm, or with both, thus preserving for ever the new malignant neoplastic property. From a theoretical point of view, then, the general idea of virus infection has much to commend it, though it cannot be regarded as intellectually satisfying in its present state of development. One can merely say that there is some factual evidence in support of it. From the purely medical aspect also the idea is convenient. Deliberately planned therapy would aim at antagonizing the immediate cause of cell division, that is, at the destruction of intracellular virus. All available medical resources might be concentrated on searching among antibiotics for one effective against hypothetical tumour viruses. Antibiotics exist which are non-injurious to cells while they are inimical to bacteria, and even to some cytoclastic viruses, e.g. psittacosis. Thus there would be very great hope of solving the problem of cancer therapy without necessarily first identifying the continuing cause of the disease. With so much to be gained from acceptance of a virus or viruses as the cause of all tumours, the evidence for and against has been thoroughly assessed in previous editions. It now remains to bring this assessment up to date.

Despite many unsubstantiated hopes and claims, it is still true that tumours caused by viruses have been found in only three classes of animals and one plant. The animals include many avian chicken tumours, one in the duck, and the many fowl

leucoses ; one tumour of one amphibian, the renal adenocarcinoma of the leopard frog ; there are three virus tumours of two mammals and some infectious warts of human beings. The mammalian tumours are the Shope papilloma and carcinoma of wild cotton-tail rabbits, and the carcinoma derived from the same virus when it is inoculated into domestic rabbits. Finally, the last tumour virus to have been found is the Bittner agent of heritable types of mammary tumour transmitted in the milk of high cancer strains of mice, to which extensive reference was made in the last edition. The first of all these viruses, that of the Rous sarcoma, was sought for and found by the classical methods of grinding tumours with sand and diluent, filtering to separate cells from fluid and small particles and testing cell-free filtrate for tumour-provoking capacity. Anyone now wishing to lay claim to the discovery of a new tumour virus would do well to refer to Rous' original papers (1911). It remains essential and obligatory to make a physical separation between viable cells and hypothetical virus at some stage of the experiment, or to destroy and to prove beyond doubt that any cells present in an inoculum are no longer capable of multiplication under the best conditions that can be provided. Some competence in elementary histology is needed. With the greatest care and caution, and also regard for all previous experience, Rous ensured this physical separation at the outset of his experiments. The same condition has been fulfilled eventually for all proven tumour viruses. A comparable separation may be made instead at a later stage ; for example, when crude tumour extract is injected into the peritoneum. This membrane will then achieve a separation between minute particles of virus and tumour cells. Virus particles will enter the blood-stream either directly or by diaphragmatic or other lymphatics, or they will be carried across endothelial membranes by phagocytes. They will not be followed by tumour cells ; or if a few of these do get through after the manner of india-ink particles (MacCallum, 1903) they will be carried to and arrested in the lungs. Virus particles that enter the blood-stream will next reach and infect those cells for which they have a special affinity. In this way the agent of Lucké's leopard frog renal adenocarcinoma was first classified as a tumour virus. The experimental transmission of Bittner's agent is frequently achieved by intraperitoneal inoculation of a relatively crude supernatant fluid after spinning. It localizes at a distance in the mammary epithelium as a pathogen.

Since Rous' original discovery, advances in knowledge of

tumour viruses have taken two directions. The properties of the viruses themselves have been sought on the one hand in optical, chemical, and physical terms; on the other, in virus-host relationships. These include histopathology, serology, the observation of conditions of infectivity, of progressive growth and of latency. Little has been added to what Rous achieved in a few years with the chicken tumour agent.

Chemical and physical properties of viruses are of greater interest to the specialist than to the general reader. They will therefore be referred to only briefly with a guide to sources of information. The biological properties of all the tumour viruses are associated with nucleo-proteins of high molecular weight. Thus Bittner's agent and the virus of yellow fever have molecular weights between three and four million, i.e. 100 times the weight of egg-albumen molecules. Physical properties include estimates of size in diameter and volumes of particles, of sedimentation rates and particulars of filterability. Diameters are now expressed in Angstrom units rather than micro-μ (for conversion of former data: 1 Angstrom = one ten-thousandth of a micron). Temperatures at which viruses are killed and at which they survive have been measured. The Bittner agent of heritable mammary tumours of mice, for example, is killed when held at 61° C. for thirty minutes. All the agents survive freezing at very low temperatures and drying in the frozen state. They are destroyed by ultra-violet radiation but survive X-radiation. Recently acquired data is available in two publications of the American Association for the Advancement of Science: "A Symposium on Mammary Tumours in Mice," No. 22, 1945; the Research Conference on Cancer, 1945; in "Les Ultravirus des Maladies Animales," Paris, 1943, edited by Levaditi, Lejeune and Verge, and the "Annual Review of Biochemistry" (1949).

Optical Properties. Electron microscopy of viruses has, for the time being, replaced other attempts at visual observation of them and is developing rapidly. As is always the case when entirely new techniques are introduced, normal appearances are in need of interpretation before the pathological can be defined. Considerable hesitation among authors has naturally been evident before committing themselves to an opinion on the nature of unfamiliar structures in unfamiliar pictorial representations. Viruses may be photographed free or associated with other particulate matter or inside the cells they infect. When free, it is by no means certain which of a variety of particle sizes is the one

that may be derived from tissue elements alone or combined with virus. Previously unidentified particles seen within cells are not necessarily viruses. With these provisos in mind, and with regard for the immature state of the subject, the reader may safely be directed to some biophysical studies of Passey, Dmochowski, Reed and Astbury (1950), and to a review by Barnum and Huseby (1950). These studies were made on extracts of tissues of high- and low-breast cancer strains of mice. They concern electron microscopy and ultra-centrifugation of particles which probably contain among them the infective agent (Bittner's milk factor) of heritable mammary tumours in mice, to which extensive reference was made in the previous edition of this volume. The particles photographed varied in diameter from 200 A to 1,200 A. They were found in large numbers in extracts of mammary tumours and in the milk of high cancer strain mothers. They were seen occasionally or not at all in extracts of normal tissues and in milk of low cancer strains. The relationship between these particles and a tumour inducing factor (Bittner's agent) can only be established by the appearance of mammary tumours in breeding mice into which they have been injected previously, for preference before or around puberty. If used for test at a later stage, multiple injections must be made. The mice themselves must be free of agent. The test is a lengthy one and these authors' final results are still awaited.

Electron microscope photographs of structures which may represent intracellular Bittner agent have also appeared (Porter and Thompson, 1948). These were made from mammary tumour cells grown in tissue culture and showed, scattered or packed in some of the epithelial cells, spherical particles of characteristic density and morphology which distinguished them from normal particles. Their diameter was estimated to be 1,350 A, that is, larger than the biggest and more than four times the diameter of the majority measured by Passey and his colleagues. Control observations on epithelial cells derived from non-heritable mammary tumours free of Bittner's agent were promised, but have not yet appeared. The practical importance of these studies lies in the possibility they open out for the detection of similar particles in human tumour extracts and milk. Similar studies have been made on the Rous sarcoma agent (Claude *et al.*, 1947).

Specificity of Tumour Viruses. All tumour viruses so far investigated have shown an almost exclusive species and cell specificity as one of their more striking characteristics. The

Rous chicken sarcoma virus itself presents certain exceptions to exclusiveness of cell infectivity. A great variety of tumours arising from many different types of connective tissue cell has been obtained with it in ducks, turkeys, guinea-hens and pheasants, in very young animals (Duran-Reynals and Shrigley, 1945), and many fowl leucoses change their morphology. Yet under the usual conditions most of the fowl sarcoma viruses will infect only connective tissue cells of a particular kind—the Shope papilloma virus will infect only epidermal epithelium ; Lucké's virus, the epithelium of the leopard frog's kidney ; and Bittner's agent is pathogenic only in mouse mammary epithelium, but is carried as well in the blood and some organs. The strictness of this cell specificity is well illustrated by two kinds of rabbit papilloma, both of them due to virus agents having distinctive antigenic properties. The oral papilloma virus will infect only buccal mucosa, Shope's papilloma virus only susceptible epidermis of rabbits (Parsons and Kidd, 1936–37 ; 1943). Conversion of mucous membranes into keratinizing epithelium by vitamin A deficiency failed to allow the Shope virus to " take " on it. Agent and anatomical site are not interchangeable in these infections. The oral virus is further restricted in action to the under-surface of the tongue. On no other variety of mucous membrane, and rarely on the dorsum of the tongue, is it possible to induce these papillomas.

According to some fervid supporters of the general application of the virus hypothesis of cancer, a mammary tumour virus, possibly Bittner's, is an exception to the rule of cell specificity owing to the frequent association, especially in pure line strains of mice, between mammary tumours and sarcomatous transformation of the stroma, the idea being that the epithelial adapted virus passes to and infects connective tissue cells. This subject is discussed on p. 138.

Virus-Host Relationships. The conditions under which tumour viruses are infective and pathogenic are of more immediate concern to pathology. In Rous' original studies (1912) he and others observed facts that led them " to suppose that the filterable causative agent requires for its action a cell-derangement or proliferation, such as a needle prick or the presence of dried tissue induces " in subcutaneous tissue. Their attempts to determine the precise stage of tissue injury or proliferation which favoured the occurrence of a tumour did not give clear-cut results. Yet these experiences and later work with the Shope papilloma

virus led again to the view that it is the young growing cell that is susceptible to virus infection and favours combined activity with it, resulting in tumour growth (Friedewald, 1942). The Shope papilloma virus will " take " only in injured epithelium either by direct application to the injury or *via* the blood-stream, from which it will localize in cuts, scratches, and the like. It will settle also in skin surfaces that have been tarred, and here too it may be doing so in cells that are proliferating in response to tar (Rous and Kidd, 1938 ; Kidd and Rous, 1938). The idea has gained support from the facts disclosed in relation to infection of mammary epithelium by the Bittner agent of heritable tumours of mice. This epithelium is most readily infected in young sucklings, though with increased dosage of agent the epithelium of older mice can be infected. During the whole reproductive life of female mice mammary epithelium is subject to formative stimuli resulting in the production of new young cells. This is perhaps the factor which allows infection, though alone it does not necessarily permit combined activity. For combined activity, as revealed by tumour growth, two more factors are required, susceptibility and stimulation by the œstrogenic ovarian hormone. The nature of susceptibility is still unknown apart from the fact that it is inherited in the manner of a Mendelian dominant in high cancer strains and occurs occasionally at random in other strains. Although non-susceptible strains of mice may carry Bittner's virus in blood and tissues, mammary tumours of the Bittner agent type and age incidence rarely develop under natural conditions. Nevertheless, infection of mammary epithelium is presumed because the agent is transmissible to susceptible young in the milk of these tumour-free carriers. Proof of the presence of agent in the milk is obtained when the susceptible young which have imbibed it become breeders and themselves bear tumours though their mothers do not. Thus susceptibility in the sense that it relates to ability to grow tumours appears to depend on capacity for combined activity rather than on mere cell infection. The other essential factor, natural œstrogenic hormone or a substitute is needed, probably in quantitative relationship with virus to get the tumour response. Some unpublished experiments of the author, in which equal quantities of tumour extract were injected into young RIIIX females (deprived of Bittner's agent) and the females then ovariectomized at six weeks' old supported the idea of a quantitative relationship. A single dose of œstrone, of about 160γ, administered through the skin, appeared to be

needed for a 50 per cent. response measured in mammary adenoma or nodule incidence. A much larger amount in a single dose appeared necessary to elicit malignant tumours. Smaller doses given repeatedly may be more effective.

Latency of Virus. Another baffling problem common to all the known tumour viruses is that of so-called latency. This latency or masking of virus is a separate problem from that of latency of potentially neoplastic cells referred to in the section on chemical carcinogenesis. Both cytoclastic and tumour viruses have the capacity to lie latent, and from this knowledge it is an easy assumption to make that when a tumour virus is not detectable it is in a latent state. Behind this assumption much of the virus hypothesis is entrenched. It is even easier to dismiss the assumption by saying that no virus in fact exists if it is not detectable in the traditional way. The strange conditions that allow detection of the Bittner virus provide a warning against this facile retort. It is doubtful if anyone in search of a virus of mammary tumours of mice would have constructed in advance of patiently acquired knowledge, the necessary conditions for its tumour-inducing activity, involving first the inbreeding of tumour-bearing and tumour-free mice, next the selection of pure line breeds of both, for all of which we are indebted to the geneticists of the U.S.A. ; next the incrimination of the mother as carrier of the tumour influence by reciprocal cross-breeding of parents from these low and high strains, and the detection of the means of transmission as being in the mother's milk ; finally, the breeding of the sucklings of infected milk until tumours arise in their mammary glands as they age. Many a mammary tumour of stock mice must have been ground up and injected, after separation from cells, into other stock mice in the early days of cancer research, without one successful transmission by virus, owing to non-observance of the necessary conditions then unsuspected. Yet it is possible that an agent either comparable to or identical with Bittner's was often present and failed to infect and cause tumours only because these conditions were not provided. Imagination, duly subject to reality-testing, rather than persistence with classical techniques, appears to be needed for detection, as distinct from verification of a virus origin of tumours. Facts that suggest that viruses may be present in a " latent " or masked state are the alternating period of filterability and non-filterability of chicken tumours and the indirect serological evidence of presence of virus in states of non-filterability.

Tumours are termed non-filterable when they cannot be transmitted by filtrates alone in the absence of cells. It has happened to many experienced workers that tumours which could not be transmitted at first, became " filterable " after several cell transplantations, or a tumour formerly transmissible in the absence of cells suddenly lost this property, yet the tumours themselves continued to grow and to look identical in either circumstance. Virus may nevertheless be detectable by serological means in non-filterable tumours. A striking example of this kind of evidence was obtained by Gye and Foulds (1935–36 ; Foulds, 1937). They were unable to transmit tumours induced with dibenzanthracene by means of filtrates, but when rabbits were immunized either with such filtrates or with the tumour itself, the serum was found to contain an antibody which neutralized the Rous tumour virus. The authors interpreted this as proof that the dibenzanthracene tumour must also have contained virus. Andrewes (1936) performed a similar experiment in which a tar sarcoma of a fowl was transplanted into pheasants, with the unlooked-for result that in two birds it grew for seven weeks to a considerable size. The serum of both these birds and of others in which regression occurred much earlier, was found to neutralize Rous sarcoma virus. The serum, neither of normal pheasants nor of pheasants immunized with fowl embryonic tissue, has ever been found capable of neutralizing this virus.

In our previous edition reference was made to the curious fact that, although carcinomas of the skin of domestic rabbits may be induced with the virus of Shope's papilloma, these tumours are only rarely transmissible by cell-free extracts (Shope, 1935 ; Selbie, 1946). Transmission by cell grafting, on the other hand, was achieved by Kidd and Rous (1940) with their V_2 carcinoma, derived from the papilloma virus, over a period of ten years. During the first twenty-five successive tumour generations, extending over more than five years, antigenic evidence of the presence of virus in the grafts was regularly obtained in serological (antiviral and complement-fixing) tests. After a further five years the same tests failed to disclose the presence of this virus. Kidd (1948) asks whether " it can be that the papilloma virus went along with the carcinoma cells merely for a ride, so to speak, playing no very essential part in their activities during its long association with them," and answers that he thinks " this not unlikely for the V_2 carcinoma cells are in the absence of the virus precisely what they were in its presence, so far as can be

told from their appearance and behaviour, while the fact is well known that non-neoplastic viruses may thus ride along in tumours." By his pertinacity Kidd has first supported, then undermined confidence in unconditional acceptance of the validity of, or need for, the idea of latency of virus, in the view that viruses are the continuing cause of cancer and in previous interpretations of serological work with chicken tumours. If for no other reason than these, Kidd's experience must make one ask what tumour viruses are doing, besides themselves multiplying, in the cells they inhabit.

Agent-free Mammary Tumours of Mice. It is generally agreed that almost no mouse strains are entirely free of mammary tumours if breeders survive long enough for these to appear. Even when mice of pure lines with a previous high incidence of mammary cancer have been deprived of the Bittner agent by cross-suckling a small number of mammary tumours will still arise, but usually at a later age than those due to Bittner's agent. These late appearing tumours are either adenocarcinomas indistinguishable in morphology from the heritable Bittner agent tumours, or vary slightly like Andervont and Dunn's Type II (1948), or are purely squamous-celled tumours, or are mixed glandular and squamous growths. Proof of their freedom from the Bittner agent has been sought and obtained by what is called the biological test and also by electron microscopy. The biological test consists in grinding the suspected tumour with diluent and injecting it into young susceptible female mice which are themselves free of the agent. This is now usually done in the first filial generation of susceptible hybrids, the female in the mating being derived from an agent-free strain. It may in future be expedient also to use male parents from a strain freed of the agent, but providing susceptibility to the cross, since Andervont and Dunn (1948) found that infected males occasionally carry agent in the seminal vesicles and may transmit it to the females. The young, susceptible, hybrid test females are then inoculated and allowed to breed. They are kept for as long as they will survive or until they develop tumours. Trials of this sort are at present in progress in many centres to test for agent in these residual spontaneous mammary tumours and also in chemically induced tumours of the gland in proven agent-free stocks. No evidence of a virus having the characters of Bittner's agent has yet been disclosed in those spontaneous or induced mammary tumours that have been tested, in sub-strains deliberately freed of the agent (Heston *et al.*, 1950).

A considerable number of these chemically-induced and some spontaneous tumours have been subjected to both biological test and electron microscopy without detection of agent (Passey *et al.*, 1950). Investigations into the origins of mammary tumours of mice have thus diverged. The Bittner agent has been characterized as a tumour virus ; its optical, physical and chemical characters and its host and hormone relationships are being investigated. Human mammary tumours and milk are being examined for particles of similar shape and size. The residual late-appearing virus-free tumours, which resemble the generality of tumours and are not heritable in very high percentages, have become a focus of investigation, both in the U.S.A. and in Europe.

Conclusions

From a theoretical point of view, the idea that tumours are caused by viruses or living particulate agents has many attractions. Examples in three classes of animals provide factual evidence of the causative *rôle* of viruses. Aided by simple injury or other special conditions probably entailing the production of young growing cells, these agents enter the young cells and therein multiply. When the cells divide, virus particles are distributed to daughter cells and thereafter to all descendants. In this way the need for an intracellular stimulus that multiplies and is passed on by linear transmission in somatic cells is provided for. Yet in stating this factual evidence and its advantages the idea of a viral impetus suffers a misleading condensation and simplification. All mention is omitted of the disappearance of virus from tumours whose behaviour and morphology is in no way modified in its absence. The serological evidence of the presence of supposedly "latent" virus was valid until Kidd reported its loss from his grafts of carcinoma induced with the Shope papilloma virus. Condensation fails to stress the cell and species specificity of tumour viruses. The cell specificities appear to be exclusive and the very rigidity of this exclusiveness makes necessary one of three assumptions. Either suitable virus agents for every type of cell are ubiquitous in nature, or they can be created *de novo* from some normal cell constituent, or virus mutations must be invoked. The very great number and variety of viruses that would be needed has always been a stumbling block to acceptance of the idea of ubiquity and multiplicity of tumour viruses by pathologists in touch with human tumour diagnosis, on account of the infinite variety of growths seen in daily experience. This particular aspect

of the virus problem is discussed by Smith and Rous (1945) in connection with their production from embryo tissues of a great variety of tumours with methylcholanthrene. They consider that the findings as a whole render it impossible to suppose that the neoplastic potentialities possessed by transplanted embryo tissues are due to the lodgment in them of tumour-producing viruses as specialized in their effects as those now known, or of precursor agents conferring neoplastic liabilities specialized to the same degree. The next of our assumptions, namely, of virus creation *de novo* from cell constituents has yet to be proved. Mutations of tumour viruses remain to be explored. Over-simplification of the issue arises from the fact that no knowledge yet exists concerning the mode of action of these agents. How is it that virus-infected cells are impelled to divide? Some accept the magical fact as intellectually satisfying without need of further enquiry (Andrewes, 1950). Others with greater curiosity will wish to know whether the mere disturbance caused by virus multiplying in the cell cytoplasm and nucleus is an adequate stimulus to division or if the stimulus is due to elaboration by virus and cell together of some specific intracellular metabolite which might arise in other ways as well as through the virus. Since an *impasse* has been reached in direct attempts to demonstrate viruses in the generality of tumours, it seems advisable to attack the whole tumour problem at any points where it will yield information. Prejudice against the idea that formative viruses may cause and perpetuate the growth of tumour cells has passed away. Viruses are the only continuing causes that have in fact been isolated. What is demanded now is rigorous proof of all claims to have demonstrated the existence of new tumour viruses and vigorous attempts to determine the characters and functions of those we know.

SARCOMATOUS TRANSFORMATION OF CONNECTIVE TISSUE CELLS

The occasional association of sarcomas with mammary tumours of both human subjects and mice, together with their more frequent occurrence during transplantation of adenocarcinomas of mice, led to much debate in the early years of experimental cancer research. The reality of the sarcomas was questioned, also the relationship whether fortuitous or causal. The ground was first cleared of the possibility that carcinomas were exhibiting polymorphism and merely resembling sarcoma cells, or that the

connective tissue change was granulomatous in character. As a result of critical morphological studies it became generally accepted that the sarcomatous transformation of connective tissue was genuine. An account of this early work appeared in Woglom's valuable "Study of Experimental Cancer" (1913). The act of propagating mammary tumours itself increased the number of sarcomas that were found to be associated with epithelial tumour grafts, and raised another question which asked whether the sarcomas were derived from graft or host. It was widely held that the malignant epithelial cells exerted an influence on the stroma of the host, sometimes converting host cells into sarcomas. Russell's extensive series of experiments (1910) led him to conclude that prolonged contact of host connective tissue cells with the grafted carcinoma was the conditioning factor in this conversion. He could at will induce sarcomas merely by leaving the malignant mammary grafts in their hosts for about sixty days, or he could maintain the propagated carcinoma unmixed with sarcoma by grafting at shorter intervals of thirty days. The idea that carcinoma cells may stimulate fibroblasts to growth and sarcomatous transformation prevailed and has received support in recent years. Ludford and Barlow (1945) observed this change in tissue cultures of mammary tumours derived from pure line mice which are specially liable to it during the course of grafting. In these tissue cultures the connective tissue accompanying the tumour inoculum is the only source of stromal cells and it may be carried along for many generations. Sarcomatous transformation, as judged by properties developed *in vitro*, was detected in six cultures and occurred once in the eleventh subculture. Final proof of sarcomatous change was not sought for by inoculating the morphologically changed cells into mice. Nevertheless, these observations make it probable that the associated connective tissue tumours may be derived from graft as well as host during animal propagation. The idea of an influence emanating from the carcinoma received support.

The subject did not come into prominence again until a suggestion was made that the influence might be a virus or living agent which passed out from the epithelial tumour cells to infect cells of connective tissue, thus provoking these also to continuous multiplication. The idea was in conflict with all previous experience of the specificity of cell infection by tumour viruses, yet it had to be entertained, more especially after the discovery that a filterable agent, Bittner's milk factor, was

responsible for heritable mammary tumours in mice, and that during the propagation of these very tumours sarcomatous transformations occur more frequently. An astonishing contribution was next made to the subject through attempts by Earle (1942–43) to induce malignant change in fibroblasts grown in tissue culture with methylcholanthrene. It happened that not only did those normal tissue cultures which were exposed to the carcinogen undergo sarcomatous transformation, behaving as malignant growths on re-inoculation into mice, but so also did most of the untreated controls, though at a slower rate. It must be observed here of cancer research in particular that very often more is learned from control experiments than from many of the ideas that give rise to them. The obvious, but not necessarily true, explanation was that contamination of glassware and tools with undetected carcinogen accounted for the change in control cultures. A concentration of 1 microgramme of carcinogen per cubic centimetre was found sufficient in the treated culture to induce malignant change more rapidly than in the controls. Other interpretations invoked virus contamination of either control cultures alone or of both these and the treated cultures, virus being assumed to be ubiquitous. Belief in virus infection was next fortified when Gey and his associates (1949) in Baltimore reported that tissue cultures of rat fibroblasts grown for two or more years in a medium presumed free of carcinogen had given rise among their progeny to several morphological varieties of cell breeds, three of which, on inoculation back into rats, led to the growth of sarcomas. The majority of normal cells grown in tissue culture do not survive transplantation in spite of an analogous immortality shared with tumour cells when appropriate conditions are provided. The cause of immortality of cells in tissue culture must be assumed to be due to the presence of mitotic stimuli among the nutrient substances reaching them at each renewal of the medium. Identification of these stimuli would probably be of great value to tumour pathology. They need not necessarily be distinct and different from the nutrient substances themselves, hence the difficulty of detecting them. It is to be presumed that such hypothetical substances are not available without cease in the animal body, else all cells sensitive to them would multiply continuously, as tumour cells do, and this is not the case. Normal cells of the body multiply intermittently in response to some known conditions, though few of the actual stimuli have been identified (Pullinger, 1949).

Ordinarily, tissue culture cells transferred back to subcutaneous tissues either perish or fail to multiply, showing that they do not there find anything that impels them to continuous reproduction.

To the author it seems that neither carcinogenic chemical of known composition nor virus infection need be assumed as the only possible explanation of sarcomatous transformation *in vitro* or *in vivo*, though they are the only ones we know of. All the evidence suggests that connective tissue cells, especially those of rats, readily undergo malignant change in response to a great variety of stimuli, frequently also in the absence of apparent cause. Two of the sarcomas which developed from carcinoma cultures in Ludford and Barlow's experiments were derived from RIII mice infected with Bittner's milk factor. Nevertheless, when mice of this strain are deprived of this agent, and thus of their epithelial tumours also, sarcomas arise spontaneously and independently in subcutaneous connective tissues of these agent-free sublines (Pullinger, 1952). Transformation of normal connective tissue to sarcoma cells *in vitro* provides a challenge to the research worker no less great than the spontaneous appearance of sarcomas *in vivo*.

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CHAPTER VII

THE CAUSATION OF CANCER: FURTHER CONSIDERATIONS

SOCIAL AND RACIAL FACTORS IN CANCER

The influence of hygiene, occupational environment and social status on the incidence of cancer has been the subject of a fascinating series of papers by Kennaway and his co-workers (1947, 1948, 1949).

Cancer of the Lung. This disease has increased sixteenfold from 1921 to 1945 in males (in England and Wales), whereas cancer of the larynx showed little change.

In attempting to explain this great increase of lung cancer Kennaway came to the conclusion that "although the incidence of lung cancer on those occupations where there is exposure to road dust (bearing in mind tarred roads) . . ." is rather above the general level, it seems very questionable whether the recent increase in this disease among the general population can be attributed to the tarring of roads. For scores of years before this increase began coal-tar was being discharged into the atmosphere in the form of soot by the domestic fire in quantities vastly greater than any "that could now be derived from roads. Tarring of roads has undoubtedly increased the amount of dust derived from tar in the air, but this increase appears negligible in comparison with the amount of soot already present." "Cotton mule-spinners show an especially small liability to cancer of the lung, although they inhale air sprayed with an oil which produces cancer of the skin."

"The high mortality from cancer of the lung in towns (Stock), the low mortality in agricultural occupations, and the absence of social gradient (Stevenson) are compatible with an ætiological factor in the air such as coal smoke."

Kennaway (1947a) is led to conclude that "coal smoke does not account well for any recent increase in cancer of the lung." "One must look, therefore, for some other factors to explain the increase, if there is a real increase, in cancer of the lung in recent years. Many such factors have been suggested, e.g. influenza, tobacco, popular drugs, tarred roads, exhaust gases and other emanations, including lead ethyl, motor vehicles

driven by petrol or Diesel engines."[1] It might be added that experiment shows that carcinogens can act remotely from the point of application ; for example, urethane causes lung tumours in young mice *via* the placenta, and in adults by subcutaneous injection.

Cancer of the Penis and Scrotum. Enquiry has revealed that the practice of circumcision protects against cancer of the penis, for the disease is unknown among Jews, who are circumcised on the eighth day, whereas (as practised by Moslems) postponing the operation until between the third and fourteenth year reduces the degree of protection. Kennaway (1947b) says, " Cancer of the penis is very prevalent among some peoples of Asia who do not practice circumcision," and, discussing the cause of the immunity evoked by circumcision, states : " Perhaps the most probable, and the most interesting interpretation . . . is to suppose that the train of events leading to this malignant growth is set going in the earlier years of life, and that removal of the cause does not then avert the final appearance of cancer at a much later age."

Cancer of the Uterus. Kennaway (1948), in an impressive paper detailed by thirty-four tables of data, comments that " all of the collections of data (material from London, Munich, Amsterdam, Rotterdam, Vienna, Budapest, Sweden, Palestine, New York, Chicago, Rochester and Philadelphia) which have been found in the literature show an incidence of uterine cancer which is greater in non-Jewish than in Jewish women." " The low incidence of cancer of the uterus in Jews is the more remarkable in that they are subject to some conditions (early marriage and child-bearing ; in some communities low economic status) which in other peoples appear to increase the liability to this form of cancer." " Various degrees of isolation of the menstruating woman are, or have been, practised over a large part of the world. The Jewish ritual appears to be the only one which imposes an exact test for the cessation of flow after five days, and for its possible recurrence during the following seven days. This twelve-day period of abstention from intercourse is of interest in regard to what is now known of the usual time of ovulation."

Kennaway finds that the data he has collected " Suggest the existence of two factors which may increase the incidence of cancer of the uterus, namely :

[1] Cigarette smoking is now thought to be a very important factor in the causation of lung cancer.

"1. A factor which is opposed by the Jewish practice of abstention from intercourse during most of the first half of the ovulating cycle ; and

"2. A factor which is intensified in both married and single women by descent in the economic scale."

Cancer of the Skin and Lip. Kennaway, in an analysis (Kennaway *et al.*, 1949) of the incidence of cancer of the skin and lip (nearly 6,000 cases) in males over fourteen years of age in a large number of occupations based on the 1931 census, and the Registrar-General's reports for thirty-four years (1911–44), states : "The ratios of the population producing one death for cancer of the skin, or of the lip, namely :

	Agricultural	Mining	Professional Occupation
Cancer of skin . .	1·0	·1·5	3·0
Cancer of lip . .	1·0	1·8	12·9

indicate that in cancer of the lip, some anti-carcinogenic factors, very much more powerful than the protection from sunlight which the miner's life affords, are active among the professional classes."

Other factors to be taken into consideration are the possible neglect of symptomless lesions which is more likely to occur in unskilled workers and labourers, "and also the susceptibility of the lip to defective supply of vitamin B_2 (riboflavin)."

Cancer of the Scrotum. Studying the records of cancer of the scrotum in England and Wales over a number of years, Kennaway found that "Cancer of the scrotum provides the extreme instance of the effect of social status on the incidence of cancer of exposed sites." "One case only of cancer of the scrotum occurred in England and Wales in thirty years in seventeen occupations of the highest professional class ; this one case was that of a person who in earlier life had belonged to a lower social class. The number of cases to be expected among the same number of persons, not specially exposed to carcinogenic materials, in the general population, would be about twenty-two."

In the years 1930–32, in round figures :

A quarter of a million men in the highest social class (I) gave no cases of scrotal cancer ;

One and a half millions in Class II gave 16 cases ;

Five and a half millions in Class **III** gave 96 cases ;
Two millions in Class **IV** gave 36 cases ;
Two millions in the lowest social class (V) gave 59 cases.

Kennaway has suggested that cancer of the penis could thus be eliminated by early circumcision, and cancer of the scrotum could be prevented by the hygienic habits of the higher economic strata of society.

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Dietary Changes and Carcinogenesis

An immense amount of investigation has been done on the effect produced by changes in the quantity, composition and calorific value of the diet, on the incidence of spontaneous and experimentally induced cancer in animals.

Many of the numerous publications on this subject deal with the effect on tumour induction of the addition, or withholding from the diet, of substances like egg-white, biotin, riboflavin, methionine, yeast, vitamin B, or dietary fat ; some of the papers make depressing reading, for they give the impression of being rather mechanically designed.

Noteworthy experimental work in this field has been done by Tannenbaum (1940–47), who concludes that a diet which keeps the experimental animal in an under-nourished state (and consequently undersized although healthy) will also reduce the development, nutrition and growth of its tumours, whether spontaneous (mammary) or induced by carcinogens. Tannenbaum's method is to feed the animals on a diet made deficient in calories by decreasing the carbohydrates. His results can be summarized in tabular form as shown on opposite page.

Tannenbaum has also reported that decreasing the food supply from 3·8 to 2·5 gm. per diem reduced the incidence of spontaneous hepatoma in male C_3H mice from 44 per cent. to nil. The numbers of mice were 50 and 21 and the diet consisted of Purina fox chow meal 40 per cent., skim milk powder 20 per cent. and corn starch 40 per cent. The mice fed on the ample diet weighed 37 to 40 gm., and those on the restricted diet 22 to 24 gm.

TABLE 10

Experiments of Tannenbaum showing the Effect on Tumour Production of Reducing the Calorific Value of the Diet

Composition of Diet			Ash	Total gm. Fed to One Mouse/ Diem	Calories Consumed/ Diem	Tumours		Carcinogenic Process	Average Weight of Animal in gm.
Protein	Fat	Carbohydrate				No.	%*		
17	2	67	4	3·4	10–12	26	54	Spontaneous mammary tumours in virgin dba mice.	30
25	3	57	6	2·3	8·2	6	12		22
29	3	52	7	2·0	7·1	0	0		20
20	2	63	5	3·3	11·7	22	85	Spontaneous mammary tumours in parous dba mice.	28
29	3	52	7	2·3	8·1	9	37		22
16	2	69	4	4·2	13–14	35	65	Skin tumours induced with benzpyrene; dba male mice.	38
29	3	52	7	2·3	8·1	12	25		21
17	2	67	4	3·4	11–12	35	74	Spontaneous mammary tumours in virgin dba mice.	33
21	2	62	5	2·7	9·6	23	47		24
17	18	53	4	2·0	8·9	41	87		24
16	2	69	4	4·0	14·3	43	86	Skin tumours induced by methylcholanthrene.	34
26	3	55	6	2·4	8·5	7	15		20
23	18	45	5	2·0	8·5	14	29		20
22	2	60	5	2·8	10·0	8	27	Spontaneous mammary tumours in C_3H virgin mice.	23
24	3	58	6	2·6	9·2	2	7		22
28	3	52	7	2·2	7·8	0	0		18
23	18	45	5	2·7	11·9	27	90		27
23	18	45	5	2·0	8·5	5	17		20
23	18	44	6	1·8	7·8	1	4		19

* Expressing as a percentage the ratio of the number of tumour-bearing mice to the number of effective mice, i.e. those which survive to the time of appearance of the earliest tumour.

The effect of a limited diet on the development of mammary tumours in mice has also been studied by Visscher and his colleagues (Visscher *et al.*, 1942 ; Huseby *et al.*, 1945). Virgin C_3H mice were maintained on a diet containing adequate proteins, vitamins and salt, but of low calorific value by a reduction of the fat and carbohydrate. The mice fed on such a diet where the calorific value was lowered by one-third did not develop mammary cancer, whereas the control mice fed *ad libitum* showed a total of 72 per cent. mammary cancer at seventeen to eighteen months

of age. The body-weight of the mice on the restricted diet remained constant at 12 to 14 gm.; the controls weighed 30 to 32 gm. after one year. Post-mortem examination showed that the under-nourished mice had lesions of the ovaries and uteri, suggesting that the lowered mammary tumour incidence was a result of pituitary insufficiency due to inanition.

Effect of Dietary Choline Deficiency. Copeland and Salmon (1946) found that depriving rats of choline in the diet gave rise to liver cancer. In a further series of experiments these authors used a diet consisting of degerminated corn grits, water-extracted casein, sucrose, lard, salts and corn oil supplemented with cystine, α-tocopherol, carotene, calciferol, thiamin, pyridoxine, riboflavin, Ca-pantothenate, nicotinic acid and inositol. Of 18 rats fed on this choline-deficient diet, 14 showed at post-mortem neoplasm of one or more types; 10 had tumours of the lungs; 4 had adenomatous liver neoplasms; 9 had severe portal cirrhosis of the liver; 3 had sarcomas in the abdominal region or in the thigh; 4 showed pancreatic growths of adenomatous type; 1 had a papillomatous epithelial outgrowth of the bladder, and hæmangio-endothelioma were present in the mesenteric and subcutaneous fat in 4 of the 18 animals. In the 20 control animals receiving the diet just mentioned, but supplemented with 2 gm. of choline chloride per kilogram, there were no abnormalities in 19; 1 rat had a severe inflammatory condition of the lungs.

Liver Cancer in Bantus. Berman's studies (1935–37; 1940) of liver cancer in Bantu are of remarkable interest (see also des Ligneris, 1936). The native mine-workers on the Rand at Johannesburg are young adult males, aged eighteen to forty-five, who rarely remain more than nine months in the mines; 35 per cent. of these men come from Portuguese East Africa, the others are from the different areas in the Union, Swaziland, Basutoland and Bechuanaland. The majority of the liver cancers found were in the Portuguese East Africans, and it was concluded that although the differential liver cancer rate is very high (90·5 per cent. of all carcinomas), yet the Portuguese East African Bantu is six times as susceptible as the mine-workers belonging to the other Bantu peoples.

Clearly these results present problems of genetics, environment and diet which are begging for solution. Berman points out that the Bantu population of Johannesburg (urban and mine) has a much lower general cancer rate than the white population—18·8 per 100,000 compared with 89·3 per 100,000. Does the Bantu

population as a rule fail to reach the cancer age? Or has this race a general resistance to cancer? The much higher death rate (3 : 1) of the Bantu inclines one to the first view.

The diet of the South African Bantu native is deficient in that it consists largely of mealies (maize); his liver cancer incidence is far higher (forty times) than the rate for Europeans, but the negroes of North America, who must be the descendants of allied African types, have a liver cancer incidence only about twice as high as North American whites. The diet of *these* negroes probably closely approximates to that of the white populations of their localities. The South African natives share this peculiar organ susceptibility with other southern, tropical and eastern races (Tomlinson and Wilson, 1945).

Berman, in his most interesting and recently published monograph, "Primary Carcinoma of the Liver" (1950), says: "Since the disease appears to single out pigmented people, many writers have been inclined to attribute the extraordinary distribution to racial or genetic factors." "My own view, based on experimental and pathological evidence, is that sensitivity to primary liver cancer is due to environmental causes rather than to genetic factors."

Berman's summary of incidence is as follows:

1. Primary liver cancer is very rare among all Western people, irrespective of whether they live in Europe, America, Africa or elsewhere. The autopsy rate is 0·14 per cent. in Europe, and 0·27 per cent. in America. The percentage frequency compared with all forms of cancer is 1·2 per cent. in Europe and 2·5 per cent. in America.

2. On the other hand, primary liver cancer is relatively common among the Bantu races of Africa and among certain Oriental races, in some of whom it is more than forty times as frequent as in Western people. The autopsy rate for the Bantu is 1·1 per cent., and 0·76 per cent. for the Oriental races.

3. Among Oriental races the post-mortem rate is as follows:

	Per cent.
Javanese (Malays)	1·31
Japanese	0·97
Chinese	0·9
Filipinos	0·44
Indians	0·32

4. The percentage frequency of primary liver cancer to all other forms of carcinoma is as follows:

	Per cent.
(*a*) Among all the Bantu races of Africa	50·9
In young male Bantus employed on the Witwatersrand gold mines	86·8
In Bantu females	5·1
Among semi-Bantu	15·3

(*b*) Among all Oriental races	13·9
Among Javanese (males and females)	41·6
Males only	79·3
Females only	6·2
Among Chinese	33·0
Among Filipinos	22·2
Among Indians	17·5
Among Japanese	7·5

5. Primary liver cancer is almost six times more frequent in the East Coast (Portuguese East African) Bantu than in the South African Bantu.

6. The Chinese immigrants in Sumatra, and (according to isolated accounts) in some parts of the Western hemisphere, as well as Chinese born in Java, show a high incidence of the disease.

7. The incidence of the disease among American negroes, although higher than in Europeans, is very low by comparison with Africans and Orientals.

8. Over 250 cases of primary liver cancer have been reported in mammals and birds.

The primitive economic and dietary conditions of the native Bantu population has led to the consumption of a simple diet consisting chiefly of a porridge of maize pap, in some areas supplemented by fermented cow's milk. Gillman (Gilbert and Gillman, 1944; Gillman and Gillman, 1945), who has made a close study of Bantu diet states: " It is a natural consequence that deficiency diseases of all kinds, including pellagra, are widespread among the black people in South Africa." " We consider the high incidence of cirrhosis and primary carcinoma of the liver in adolescent and young adult Africans as being due in no small measure to repeated insults to the liver resulting from acute and chronic malnutrition."

Findlay (1950) observed a high liver cancer incidence in West African negro soldiers and considers that the Bantu mine workers and the West African soldiers were probably better fed when they came under observation; the damage to the liver must then be considered as having already begun to take effect. He says " In West African recruits evidence of riboflavin deficiency was so common as to be almost the rule; not infrequently there was marked crazy-pavement skin, which is variously described as being due to vitamin A deficiency or to lack of pantothenic acid. On the very complete rations supplied to African soldiers it was usually required from eighteen months to two years to ensure the disappearance of all signs of deficiency." He states: " In West African soldiers recruited in the Gold Coast and Nigeria, approximately 25 per cent. were infected with *S. hæmatobium*, but histological study of their cirrhotic livers, and of the associated primary liver carcinoma did not commonly reveal the presence of Schistosome eggs. *S. mansoni* infections are less common except in certain small foci. It is possible, however, that toxins derived from other helminths may give rise to liver necrosis. Deschiens *et al.* (1949), for instance have shown that extracts of *Ascaris lumbricoides* and *Tænia saginata* injected into guinea-pigs, rats and cats cause necrosis of liver cells. African soldiers showed almost 100 per cent. infection with *Ascaris* and under civilian conditions constant reinfection with intestinal worms is the rule."

Findlay concludes that " Of 60 malignant tumours causing death among 227,000 West African soldiers, aged eighteen to forty years, 37 were primary carcinomata of the liver. While a diet deficient in sulphur-containing amino-acids is probably the primary factor leading to the cirrhosis which appears to be a precursor of primary hepatic carcinoma, infective hepatitis, the toxins of worms, alcohol, malaria, and possibly manganese or selenium may play a secondary *rôle*."

However, Findlay and Enbliter found that rats which had been fed on a protein deficient diet did not develop liver cancer, but other investigations have shown that the induction of liver cancer in rats by butter-yellow given with the diet can to a certain extent be inhibited by supplementing the diet with protective materials like fresh milk, riboflavin, casein and methionine.

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The Hypothesis of Somatic Mutation

Somatic mutation, of all cancer theories perhaps the most widely accepted, postulates that tumour cells are mutated normal cells. The evidence rests largely on the parallel between mutating agents and carcinogenic agents, although the theory was advanced before carcinogenic compounds were discovered. But just as is the case with carcinogens, the catalogue of mutagens is increasing, with the result that some potent mutagens are found to be non-carcinogenic and some carcinogens are not mutagens. For example, two of the most active mutagens, phenol and formaldehyde, have still to be proved carcinogenic.

In so far as a mutation is a change in a cell or organism which

carries the new characters into succeeding generations, the definition is quite adequate, though it is little more than a replacement of terms.[1] A number of objections can, however, be levelled against the mutation hypothesis ; for example, conclusive proof would require hybridization of tumour cells. Moreover, mutant forms, such as the different types of Drosophila produced by mutating agents, show a whole range of morphological variations, while the carcinogen on the other hand creates a new type of cell with a single, highly characteristic property, that of neoplastic multiplication. To extend somatic mutations to include other pathological changes in tissues would not be welcomed by the protagonists of the theory ; to reserve it for carcinogenesis would revive the danger of mistaking exchange of nomenclature for explanation. A white-eyed fly mutant is born not made ; the conversion of a wild type to white-eye type, *during life*, i.e. a somatic mutation, must be a very rare event indeed, although the change can often result from treatment with mutagens *via* the germ-plasm of the previous generation. Any attempt to explain this kind of difficulty by referring to the term " somatic " can hardly prove satisfactory. Furthermore, sex hormones can change the sex of a chick without altering the sex chromosome characteristics, showing that other factors besides the nuclear structures are operative in deciding whether the tissues and cells of the body shall secrete or grow according to a masculinizing or feminizing plan. How can the morphology, physiology and other characteristics of the scores of types of cells in the body be accounted for on genetic grounds, if, as theory insists, the chromosomal structure of all the cells in the body are identical ? Any one particular mutation is a somewhat rare event, since it depends on a fundamental change in a locus on a chromosome, of which there are thousands in the four chromosomes of Drosophila, and the number is much larger in mammals. Cancer, on the other hand, probably arises simultaneously in many cells of a tissue (multicentric origin), not in one, as has been emphasized by Willis (1948) in his book, " The Pathology of Tumours," where he states :

" A skin cancer in its early formative stage arises more by general transformation of pre-existing epidermis than by cellular multiplication, and only after the formative field has all suffered

[1] H. J. Muller (1950) writes : " If, however, the mutation were such as to give the cell the tendency to unregulated multiplication a condition would arise like that found in malignant tumours and leukæmias."

neoplastic change does the tumour grow solely by multiplication. The two processes, neoplastic transformation and proliferation, overlap, the former predominating during the early genesis of the tumour, the latter often being initially negligible, but gradually taking an increasing and finally exclusive, part in the growth of the tumour" and "That mammary tumours often arise simultaneously or successively from extensive tracts of breast tissue is clear from the work of Cheatle (1921), Nicholson (1921), Cheatle and Cutler (1931) and Muir (1941). To Cheatle belongs the credit of first demonstrating this conclusively in sections of whole breasts."

Willis quotes Dawson, who says: "In the study of the advancing margins of many of the malignant melanomata, especially those which showed zones of pigmentation fading off into the normal skin, numerous appearances were observed which pointed definitely to the presence of multiple and isolated points of origin of the early malignant change . . . numerous isolated groups of intra-epidermal cells could be found undergoing a malignant transformation."

Again, when mice are treated with urethane, cancer can arise in as many as 100 different places in the lung. Finally, while mutations are rare in humans, something of the order of 15 per cent. of our species becomes cancerous. Moreover, if the affected tissue is surgically removed, the adjoining cells all too frequently undergo neoplastic conversion.

It is by no means easy to see how the same genes of the thousands present in the forty-nine chromosomes could mutate in a number of cells simultaneously. These facts raise serious contradictions for any mutation theory which depends upon changes in the chromosomal gene system.

Attempts to overcome some of the difficulties of the nuclear gene mutation theory by one which postulates an extra-chromosomal gene system, would fit in well with a virus theory where the virus can be regarded as loose genes located in the cytoplasm. Such a theory, based principally on examples of maternal inheritance and on chemical similarity of nucleoproteins and virus proteins, does not resolve the difficulties attending proof, and in fact does little more than substitute the hypothetical cytogenes for the invisible genes on a visible carrier system (i.e. the chromosomes). Here the supporters of the plasma gene theory would find themselves in deep water; according to Miller and Pybus (1945), no extrachromosomal

factor has been found for the inheritance of mouse lung tumour, hepatoma or bone tumours. The explanation that the plasma gene operates *via* the nuclear gene would take us further into what is still speculation if not guesswork.

Although much is heard of tumour cells being mutated normal cells, less is said about tumour-liable mice as being mutated normal mice. How would the enthusiastic supporter of mutation theories of cancer explain the origin of a tumour-liable strain of mouse ? He would have to suppose that *somatic* mutation led inescapably to mutation of the *genetic* material whereby the potentiality for malignancy is transmitted.

Undoubtedly there is something compelling in the title " Somatic Mutation," but the theory cannot be sustained without the aid of *ad hoc* hypotheses.

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The Influence of Heredity

It is clearly of importance to know whether an increased liability to develop cancer may be inherited. This question is being studied by experiment in animals and by observation in the human subject. Experiments show that the issues are far more complex than is often supposed and, with few exceptions, reports of human investigations are conflicting.

It now appears certain that hereditary elements contained in the fertilized ovum play a part in determining whether or not certain animals will develop tumours, but there is much confusion concerning the way these elements act in the eventual realization of the tumours. The complexity of hereditary and environmental factors, even those associated with the short-lived mouse, has been clearly explained by Heston (1944–45). Confusion arises from the assumption that all biological characters are direct representatives of single genes, the name given to the hypothetical material bases of inheritance, and that Mendel's laws which apply to these underlying hereditary elements can be applied to composite characters also. These characters, whether tumours or

coat colour, are the eventual product of gene action in particular environments. They represent the sum total not only of gene influence, but also of other heritable nuclear and cytoplasmic elements, of the genetic constitution and of the environment. They may, but do not necessarily, make their appearance in Mendelian proportions as dominants or recessives, as do those single characters that are directly related to genes. When the inheritance of lung adenomas (Andervont, 1937, 1938; Heston, 1940–41), mouse leukæmias (MacDowell, 1936), and mouse mammary tumours were analysed in homozygous strains and crosses, it was found in all of these that multiple factors were contributing. Evidence was found of both genetic contributions and of the operation of naturally-occurring or artificially conditioned environmental factors. Natural environmental factors can seldom be identified; examples of artificial ones, which raise the natural tumour incidence in some strains, are the carcinogenic chemical compounds. Food deficiencies sometimes lower it (see Table 10).

Some of the numerous factors which are effective in the development of hereditary mammary tumours of mice have been sorted out. They serve to illustrate and emphasize the complexity of multiple factor inheritance. Homozygous mouse strains have been bred in which the incidence of breast cancer is 100 per cent. In others this tumour is non-existent or very rare. Between these extremes there are all degrees of incidence, a variability which itself points to multiple factor inheritance. As recounted in the previous edition, one of the essential factors is a virus passed on to the young in the mother's milk. Thus transmission of this tumour is extrachromosomal. Another is the hormonal make-up of the animal. Tumour incidence is dependent upon natural œstrogen, or in males on artificially applied œstrogen. Finally, a factor known as susceptibility is essential and operates in some strains like a Mendelian dominant. This susceptibility has been further analysed. It consists of at least two components. One is a receptivity or responsiveness in respect of the Bittner agent (milk factor), probably at focal points in the mammary gland, the other is again hormonal in nature. For example, virgin female mice of one strain containing the agent will develop tumours, while those of another, also containing the agent, do not. The mothers of both develop a high incidence. This suggests that the hormonal influence may be quantitative in its action, and may depend on the genetic constitution in respect of hormone

secretion in these mice, the inherited hormonal influence of Bittner (1945). Heston has expressed the opinion that if and when we describe the links leading to susceptibility, we shall be able to go back link by link to the gene, that which is actually inherited. Another example, often quoted, of inheritance of cancer under experimental conditions is melanosis in hybrids of spotted platy fish and sword-tail fish. The nature of this melanosis, whether a progressive hyperplasia or a true blastoma, might be settled by attempting to graft the large melanophores into sword-tail fish. A more malignant type has recently been recorded (Nigrelli *et al.*, 1951).

Complexities such as those described show why it is impossible for the geneticist to make predictions about tumour incidence in genetically heterogeneous human populations. From ancestry data alone it cannot be asserted that a complex multiple-factor character will appear in an individual of the progeny. Nevertheless, two human neoplasms appear to have a relatively simple mode of inheritance. These are intestinal polyposis with carcinoma and retinal neuroblastoma. Dr. Cuthbert Dukes (personal communication) has kindly provided the following commentary on intestinal polyposis :

"In most cases of rectal cancer there is no evidence of an inherited susceptibility or familial predisposition to cancer, but these are quite obvious in the small proportion of cases of rectal or intestinal cancer associated with polyposis or multiple adenomata. Familial polyposis is a rare disease. A survey of the records of St. Mark's Hospital carried out recently by Dr. Dukes (including approximately 4,000 cases) has shown that the incidence of polyposis has been only one per cent. amongst the cases of rectal cancer treated by radical excision. More than thirty families afflicted by this disease are being investigated by Dr. Dukes at St. Mark's Hospital and the evidence so far collected establishes the following points with regard to the inheritance of this abnormality. Polyposis is likely to occur equally amongst men and women and either sex may transmit the disease. When only one parent is affected the disease is likely to appear in half the children. Only those descendants of a polyposis family who manifest the disease (or would do so if they lived long enough) can transmit it to their children. In its manner of inheritance polyposis behaves as a Mendelian dominant, and in families in which only one parent is affected the distribution of this abnormality corresponds to what would be expected from the mating

of a heterozygote with a homozygote. The incidence of intestinal cancer is very high in polyposis families and malignant disease manifests itself at an exceptionally early age, in fact, at least twenty years younger than amongst the general population."

Neuroblastoma of the retina, a rare tumour with which a familial incidence is common, may arise in intrauterine life and is rarely delayed beyond adolescence. The degree of malignancy is variable, spontaneous cure by fibrosis and shrinking being known. If the eye is excised before invasion of the optic nerve, the individual may survive and thus provide an opportunity to show that this tumour is heritable. In some families it appears to behave as a Mendelian recessive or irregular dominant, in the majority it appears sporadically or in several members of one generation. The most likely cells of origin are persistent primitive neuroblasts which have retained their vegetative potentialities. This source would account for the rarity and early appearance in life of these tumours. Differentiation in the retina is complete at the end of the sixth month of life. After this none of the nuclei of the rods and cones, from which nuclear layers alone the tumours arise, divide again (Mann, 1937). In this respect the definitive functioning cells of the retina are comparable to nerve cells. Fully differentiated nerve cells do not multiply, nor do spontaneous tumours arise from them. Though many have sought to induce fully differentiated neuromas, only one author has recorded success (Russell, 1945).

New evidence concerning the incidence of human breast cancer fails to provide any suggestion that daughters who suffer from it are more likely than others to have had mothers who died from breast cancer. Many authors on the Continent still consider that a hereditary influence has been established. A method of enquiry was adopted (Passey *et al.*, 1950) to ascertain from a patient suffering from breast cancer whether her various relatives were alive or dead. If they were dead, every endeavour was made to find the town where they died and the date when they died. From this information it was possible to obtain from the Registrar-General's office a copy of the death certificate of such individuals. The figures of their enquiry are based entirely on death certificates. No notice was taken of the medical history of relatives as given by the patient. The figures thus obtained were then compared with the Registrar-General's figures for England and Wales for 1947.

As will be seen from Table 11, 23 out of the 585 mothers of

patients with breast cancer, whose death certificates were traced, died with breast cancer, and in a comparable number of unselected mothers of the same age, 20 could be expected to have died with this disease. The difference in the two figures has no significance. Similarly there is no difference in cancer of all sites in the women investigated and in the unselected figures of the Registrar-General. These figures have, however, been criticized on the grounds that the unselected mothers were derived from an age group and time that did not correspond with the deaths of the actual mothers (Smithers *et al.*, 1952).

TABLE 11

Data extracted by Passey et al., 1950. *Vth International Cancer Congress, Paris*

Mothers of Patients with Breast Cancer		Cancer : All Sites		Breast Cancers	
		Found	Expected	Found	Expected
Dead . .	585	102	100	23	20
Alive . .	199				
Not traced .	67				

TABLE 12

Controls

Mothers of Patients with Breast Cancer		Cancer : All Sites		Breast Cancers	
		Found	Expected	Found	Expected
Dead . .	277	20	47	5	4
Alive . .	148				
Not traced .	71				

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The Influence of Hormones

In attributing either benign or malignant experimental tumours to the action of hormones, it is essential to know whether certain criteria for neoplasia can be upheld. In the absence of these criteria, it is often probable that proliferations are neoplastic rather than hyperplastic, but the evidence is less secure. A diagnosis of benign growth requires that the hormone to which the origin of the tumour is attributed is no longer present in amounts capable of producing progressive hyperplasia. The reason for this is given below. A diagnosis of malignant growth requires in addition that the proliferating tissue shows some essential malignant character, infiltration or metastasis or both. It is desirable but not essential that the tumour be serially transplantable into hosts having no excess of the hormone which induced it. This last condition provides final proof of autonomy. These criteria were formerly seldom fulfilled, but increasing attention is now being paid to them. The need to exclude the effective hormone after induction has been brought about is due to the histo-specific, hyperplasia-inducing properties of these substances. Hyperplasias endure only as long as the hormones that cause them are acting. They regress when this stimulation ceases. Simple proliferation caused by hormones does not progress without limit. A certain maximum effect is attained, after which there is no progress. If the hormones are then withheld the tissue made hyperplastic reverts to something like its former state. The breast after lactation is an obvious example. Thus too, the fibroma-like tumours of peritoneum and uterus of the guinea-pig provoked by œstrogenic treatment (Lipschütz and Vargas, 1939 ; Lipschütz 1950) regress completely in the absence of excess of œstrogens. Most, but not all, hyperplasias of the anterior hypophysis induced with œstrogen revert to normal when excess œstrogen is withheld. Several points of interest arise in connection with the effect of artificially applied œstrogen on the mammary gland. It was the association of tumours of mice, especially male mice, with œstrogen treatment that first

gave this hormone its status as a carcinogen. We now have to recognize that in mice œstrogen is not effective as a mammary tumour inciter in the absence of Bittner's milk agent or in the absence of a chemical carcinogen. It is probable that when œstrogen is an effective factor in mammary cancer it is so partly because in males it brings the gland into anatomical existence and in females keeps the gland in being. Without œstrogen in adequate amount the organ would contain no tissue elements apart from atrophic ducts. In the absence of Bittner's agent, excess œstrogenic stimulation does not induce mammary tumours in mice. There is an optimum relationship between dosage and parenchymatous response ; beyond this there is no proliferation outside the bounds of simple hyperplasia and the limits set by the fat pads. It is curious that in the rat in which spontaneous mammary tumours do occur some strains respond to excess œstrogen with malignant growth (Dunning *et al.*, 1947), although nothing equivalent to Bittner's agent in mice has been described in this animal. Continuous rather than intermittent exposure to œstrogen is believed to be the effective neoplasia-inducing condition (Lipschutz, 1950 ; Burrows and Horning, 1952).

True new growths satisfying the criteria which neoplasia demands have now been induced in two ways. Either excess of a natural or synthetic hormone or combination of hormones is applied artificially from without, or the internal balance of the animal is interfered with so that one of the natural hormones preponderates. In the great majority of experiments, pure line mice and inbred rats have been used. If tumours are obtainable in one line only and not in others, the value of the result must be judged accordingly. The preponderating influence must be attributed to the inbreeding and inheritance of some peculiar response of the original mice from which the strain was derived. Since the line is a pure one, all subsequent individuals are mere repetitions of the original one or two reactors. Often, however, several strains respond in the same way. Thus Bonser and Robson (1940) and Bonser (1944) obtained testicular carcinomas in Strain A and RIII males with triphenylethylene ; Hooker and his co-workers (1940) in Strain A with œstradiol benzoate or stilbœstrol ; Shimkin and his colleagues (1941) in Strain C. One other strain and reciprocal hybrids of A have also responded. Bonser (1944) and Gardner (1945) succeeded in propagating these interstitial cell tumours in first or early grafts in the presence of œstrogen. but later independently of this hormone, thus demon-

strating the autonomous nature of some of these carcinomas. Similarly, some œstrogen-induced pituitary chromophobe tumours have been successfully transplanted (Gardner, 1947). These grafts may take up to nine months to start growing, even in mice of the same inbred strain.

Some curious examples of hormone-induced tumours occur when certain operative procedures are carried out. Firstly, if mice of several strains are ovariectomized or spayed at birth or after one week old, hyperplasia and malignant tumours of the adrenal cortex develop. Fekete and her colleagues (1941) found diffuse cortical carcinoma in four female mice of the dba strain ovariectomized on the first day of life and examined between the fourteenth and twenty-eighth months. Hyperplasia of the cortex in gonadectomized males is accompanied by development of mammary glands and by œstrus in females. If the Bittner agent is present, mammary tumours appear in both sexes, owing to œstrogen production in the hyperplastic or neoplastic adrenal.

Secondly, granulosa cell tumours, luteomas and mixed tumours have been obtained by grafting portions of either testis or ovaries into the spleens of castrate or ovariectomized rats and mice. Biskind and Biskind (1944, 1945) argued that, as œstrogens and androgens are inactivated in the liver, transplantation of the gonads into the spleen, which drains into the portal circulation, would remove the normal inhibiting influence of the gonadal steroids on the pituitary, thus permitting unopposed secretion of gonadotropin. To this excessive or unopposed gonadotropin the tumour-inducing results of intrasplenic grafting of gonads are attributed. An essential condition for success is complete removal of both gonads from their normal place in the systemic circulation of the grafted animal. These results, repeated in rats (Biskind *et al.*, 1950) have been obtained also in all strains of mice tested by Li and Gardner (1949). The autonomy of the tumours first grown in the spleens was proved by metastasis and by transplantation into subcutaneous tissue of intact new hosts. Many of these experiments point to a more direct participation of hormones in malignant transformation than seemed probable at one time, and also to a gradation of response from simple hyperplasia to true neoplasia.

In addition to direct tumour-inducing action, some hormones increase the natural incidence of spontaneous growths, for example, lymphomas and mammary adenomas and carcinomas of rats are increased by œstrogens. Hormones may convert naturally-

occurring hyperplasia into neoplasia. The peribronchial lymphosarcoma of rats induced with anterior pituitary growth hormone may be an example of this action (Moon *et al.*, 1950). Obviously, many questions remain to be solved in this wide field of investigation.

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Enzymes in Cancer

The effort to reveal the essential biochemical features which underlie neoplasia has become less prominent of late, probably because this line of research has reached the stage where further advance depends on developments in pure biochemistry, for example, in the structure and genetical significance of the nucleoproteins and in the mechanisms of carbohydrate metabolism.

In recent years the enzymes of tumour tissue and of the tissues of animals and human subjects bearing tumours has been the subject of intensive study by Greenstein (1947), who has concluded that :

"1. The enzyme systems of tumours resemble each other more than they do those of normal tissues.

2. There is more uniformity between tumours than between normal tissues.

3. The activity of each enzyme in tumours falls somewhere within the range of that enzyme in normal tissues, sometimes near the upper end of the range, more often near the lower end of the range, but never outside the normal range.
4. Malignant tissues, in comparison with normal tissues and benign tumours, are characterized not only by possessing the lowest concentration of cytochrome C, but also by possessing the greatest disparity between the components of the cytochrome oxidase-cytochrome C system.
5. Warburg's early prophecy of a deficient respiratory mechanism in tumours has been sustained by the observation of very low amounts of the cytochrome system, of catalase, of the flavin enzymes and of coenzymes in these tissues.
6. The tissues of an animal bearing a tumour show some of the enzymic properties of the tumours, i.e. the whole animal is cancerous."

I. HIEGER.
B. D. PULLINGER.

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CHAPTER VIII

THE CARDIO-VASCULAR SYSTEM

Introduction

Our knowledge in this field has been augmented in recent years by the discovery of methods whereby such fundamental hæmodynamic constants as cardiac output and coronary blood-flow can be measured in the living subject. For this reason brief accounts of modern conceptions regarding the several aspects of cardiac failure have been included in this chapter. Carditis and arteritis have also been discussed, in conformity with the growing belief that the connective tissue of the heart and blood-vessels is peculiarly liable to a series of acute inflammations which have much in common with the mesenchymal responses known to be conditioned by a state of hypersensitivity to foreign protein antigens. The mechanism of vascular occlusion in atherosclerosis and the unsuspected heavy incidence of atheromatous degeneration of the vessels of the lower limb are also described.

THE HEART

Cardiac Failure

The morbid anatomy of cardiac failure has been known for many years, but its underlying morbid physiology could not be studied until methods became available for measuring the speed and volume of the circulation. During recent years this has become possible, particularly since the introduction of cardiac catheterization. Heart failure is a complex phenomenon involving the whole circulatory system. Friedberg (1950) has defined it as a state in which the "output of the heart is insufficient for the needs of the tissues," but this cannot be regarded as synonymous with an absolute diminution of cardiac output. In healthy people the mean cardiac output at rest is 5·3 litres per minute (Sharpey-Schafer, 1944) with a range of variation between 4 L/m and 7 L/m (Friedberg, 1950). In mild grades of cardiac failure the heart's output is usually within normal limits, and it may remain so even in the presence of quite severe failure. As a general rule, however, the cardiac output is diminished and

figures about 2·5 L/m are recorded (McMichael and Sharpey-Schafer, 1944). There are, however, certain forms of heart disease, which will be discussed later in this chapter, in which the cardiac output is above the normal average even in the presence of clinical cardiac failure. It is obvious, therefore, that cardiac failure must be defined in terms of a relatively inadequate circulation.

Acute Heart Failure. Fatal cardiovascular failure may occur in three forms : acute circulatory collapse, acute heart failure and chronic congestive failure. In acute circulatory collapse, such as occurs in shock or hæmorrhage, the heart is handicapped by failure of the venous return. As a result, cardiac filling and cardiac output fall until the heart fails for lack of blood to fill it. In such cases the heart at autopsy is contracted and empty and of normal weight, whilst the great veins are relatively empty. Acute heart failure may occur because of sudden failure of the cardiac muscle, as in myocardial infarction, or because of a sudden obstruction to outflow, as in massive pulmonary embolism, or because of obstruction to the filling of the heart, as in pericardial tamponade. In the first two cases the heart fails to empty properly ; the volume of the incoming venous blood is added to the retained ventricular blood, and acute dilatation of the affected chambers ensues. This leads to a rapidly mounting rise of pressure in the great veins with consequent distension. At autopsy the heart and great veins are dilated and there is acute venous congestion of the viscera, but none of the structural changes of chronic venous congestion.

Pericardial tamponade can only cause acute heart failure if the accumulation of fluid is rapid. This is rare in pericarditis, most instances of fatal tamponade being the result of hæmorrhage into the pericardium, causing a rapid rise of pressure outside the heart which soon exceeds the venous pressure and prevents the heart filling in diastole. This leads to a fall in cardiac output, damming back of blood producing a rise of pressure in the great veins and a temporary increase in the diastolic filling of the heart. Unless relieved, the pericardial pressure rises well above the maximum venous pressure, ultimately producing failure of cardiac filling and death. At autopsy the pericardium is distended, the heart is contracted and empty, the great veins are engorged, but there is no chronic venous congestion.

Chronic Heart Failure. Chronic cardiac failure is a much more complex process because of the wide variety of causes and

the interaction of a series of compensatory mechanisms. The diseases responsible may produce increased resistance to the blood-flow or they may engender functional myocardial inefficiency. In other cases they create abnormal conditions which can only be met by an abnormally increased blood-flow. The first may be a consequence of any valvular defect, of systemic hypertension, or of pulmonary vascular obstruction. Muscular inefficiency may be due to inflammation, toxæmia and ischæmia of the myocardium. The need for an abnormally increased blood-flow has only recently been recognized and arises in a variety of conditions in which there is a greatly increased cardiac output. The most important diseases in this group are chronic bronchitis and emphysema, anæmia, thyrotoxicosis, beri-beri, arteriovenous fistulæ and osteitis deformans. In all these diseases the blood-flow is greatly increased and in time the strain on the heart is sufficient to produce cardiac failure. When this occurs the output of the failing heart, though relatively diminished, is still greater than normal. In congestive failure due to prolonged anæmia the cardiac output may be as high as 9·4 L/m (mean of 7 cases : Sharpey-Schafer, 1944) ; in chronic lung disease with congestive failure the output may be 7·7 L/m (McMichael and Sharpey-Schafer, 1944), and in osteitis deformans with congestive failure the output in 1 case was 13·3 L/m (Edholm *et al.*, 1945). In practice, cardiac failure is often the result of several separate factors. Rheumatic carditis obstructs the blood-flow by its valve lesions and also damages the myocardium ; hypertension may be associated with myocardial ischæmia, while emphysema or anæmia may complicate other forms of heart disease.

Whatever the cause of cardiac failure, the sequence of changes is essentially the same. The first effect is failure of the affected chamber to empty properly. This phase is transitory and compensation is rapid. The small volume of blood retained, added to the venous inflow, causes a slight distension of the heart which, according to Starling's law, results in more forcible cardiac contraction, bringing the cardiac output back to its normal value. At this stage the circulation will be normal, and the only evidence of failure will be enlargement of the heart and a diminution of the cardiac reserve detectable as dyspnœa on exertion. The stretching of the myocardial fibres is the stimulus for hypertrophy (Eyster, 1928). Dilatation is, therefore, followed by hypertrophy of the muscle and more stable compensation is achieved. The heart remains enlarged, but considerable reserve power may be

re-established and the patient becomes almost completely symptom-free. It is important to remember that *myocardial hypertrophy is the direct consequence of dilatation, however this may be brought about.* Since hypertrophy will occur to a maximum degree in intrinsically healthy muscle subjected to the increased mechanical load of hypertension or valvular stenosis, gross hypertrophy is most often seen in these conditions. Nevertheless, it does occur in any form of cardiac failure, even when this is a consequence of primary disease of the muscle such as ischæmia (Bartels and Smith, 1932 ; Davis and Blumgart, 1937 ; Harrison and Wood, 1949). This state of cardiac compensation brought about by hypertrophy can persist for years if the stimulus that produced it remains static, but even so the heart is working at a slight mechanical disadvantage due to the hypertrophy itself. The evidence for this is the gradual failure of a hypertrophied heart subjected to a constant, non-progressive strain, such as that of chronic arterial hypertension. It has been suggested that this is due to the relative ischæmia of the greatly increased bulk of muscle, but many authors have reported enlargement of the coronary arteries in hypertrophied hearts (Fishberg, 1937 ; Gross, 1921 ; Russow, 1936 ; Sagebiel, 1934), and Harrison and Wood (1949) found that the degree of enlargement of the main coronary arteries in hypertension corresponded with the degree of myocardial hypertrophy.

On the other hand, there is no evidence that the number of capillaries between the muscle fibres increases (Payling Wright, 1950). In this connection it must be remembered that the over-riding factor is the minute-volume of blood-flow, the actual number of vascular channels available being of far less moment. Recently, by means of catheterization of the coronary sinus, Bing and his collaborators (1949) have been able to measure the normal coronary flow in man and to compare it with the values found in various cardiac diseases. They found that the normal flow was 65 ml. per 100 gm. of tissue per minute. In hypertension the flow was 70 ml. per 100 gm. per minute, and in aortic disease they recorded normal or increased flows. As none of these observations showed a significantly diminished flow per unit of heart tissue, they afford a clear indication that the total coronary flow through a big heart is increased proportionately to the weight of the heart. They do not, however, exclude the possibility that the individual hypertrophied muscle fibre becomes ill-nourished. Myocardial fibres are nourished by the diffusion

of oxygen and metabolites from the capillaries lying along their surfaces, and since the rate of diffusion varies with the square of the distance, a relatively slight increase of fibre thickness will considerably slow up the diffusion between the capillary blood and the central parts of the fibres. Thus in an hypertrophied heart, the central parts of the muscle fibres will tend to suffer from relative anoxia even if the coronary flow per unit of muscle remains unchanged.

In most cases of heart disease compensation by hypertrophy sooner or later breaks down and the affected ventricle fails to expel its quota of blood. In the case of the left ventricle, which is most often affected, this leads to a rise of pressure in the pulmonary veins which, transmitted backwards, throws a strain on the right ventricle. The pressure in the pulmonary circuit rises, the filling pressure of the left heart is increased, and again, following Starling's law, the force of left ventricular contraction is temporarily augmented. For this increase in stroke volume the price paid is passive congestion of the lungs with dyspnœa and reduced vital capacity. Such a state of affairs is usually only temporary. After a time the right ventricle fails and typical congestive cardiac failure sets in. Venous engorgement and œdema are the outstanding characteristics of congestive failure. It has been traditional for many years to regard cardiac œdema as the direct consequence of increased capillary pressure, aided and abetted by anoxia of the capillary walls. It is now certain that both these factors, if they operate at all, play a minor *rôle* and that cardiac œdema is conditioned by retention of sodium ions and, as an inevitable consequence of this, by retention of water (Merrill, 1949).

Sodium Retention in Cardiac Failure

The exact mechanism whereby the kidney fails to excrete sodium in cardiac failure is not known. In health one of the functions of the kidney is the conservation of sodium and the regulation of water balance. The normal renal blood-flow is about 1·3 litres per minute (Friedberg, 1950), from which 130 ml. per minute is filtered off by the glomeruli—i.e. the volume of glomerular filtrate is 180 litres per day (Bradley and Blake, 1949). This volume contains 1,200 gm. of sodium chloride, of which all but about 1·5 gm. is re-absorbed by the tubules. In other words, the normal kidney tubules reabsorb more than 99·9 per cent. of the sodium filtered by the glomeruli, hence the percentage increase

of sodium reabsorption by the kidney of heart failure is an infinitesimally small one ; yet it accounts for a difference of over 1 gm. per day in the total urinary output of sodium and, since sodium chloride in the body must remain isotonic, a consequent retention of at least 100 ml. of water. One of the factors responsible is probably a diminished renal blood-flow. In heart failure renal blood-flow falls to a relatively greater degree than the cardiac output ; Merrill (1946) found that with a cardiac output reduced to one-half, the renal blood-flow was reduced to one-fifth, and similar findings are reported by Bradley and Blake (1949). The glomerular filtration rate is consequently diminished, though not to a corresponding degree. It is possible that the deviation of blood-flow from the kidney is a compensatory phenomenon which diverts the blood to the brain and other vital tissues (Bradley and Blake). The effect on the kidney, however, is to diminish the amount of sodium and water filtered by the glomeruli. This in its turn is followed by an increase in the reabsorption of sodium by the tubules. Diminished filtration alone, however, is not sufficient to account for this increased reabsorption because in other forms of diminished filtration, such as nephritis, sodium is not retained. Other factors must be considered and a raised venous pressure has been suggested by some workers (Bradley and Blake, 1949), but denied by others (Homer Smith, 1951). There is good evidence that about 85 per cent. of the reabsorption of sodium occurs in the first convoluted tubules as a passive oncotic phenomenon and the residual 15 per cent. is actively reabsorbed by the second convoluted tubule. This latter absorption is controlled by the hypothalamus and the adrenal cortex (Homer Smith), but as yet there is no positive evidence that either takes any part in the excessive sodium reabsorption of cardiac failure. Since, however, the increased reabsorption is of the order of a fraction of 0·1 per cent., it may well be that we lack methods delicate enough to detect so fine a difference.

Whatever may prove to be the final mechanism of sodium retention, there is no doubt that it is the critical factor in the pathogenesis of the œdema of congestive cardiac failure. The retention of sodium necessitates a corresponding retention of water and a state of plethora occurs with an increase of the volume, both of the circulating blood and of the tissue fluids, which in turn lead to the œdema of congestive cardiac failure. The clinical proof of this is provided by the spectacular sodium and water diuresis and the accompanying diminution of œdema, which so

often follow the blocking of sodium reabsorption in the renal tubules by mercurial diuretics.

The question of sodium retention in congestive heart failure will be briefly re-discussed in the chapter on Renal Failure.

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Pulmonary Heart Disease : Cor Pulmonale

It has long been believed on indirect evidence that the pressure in the pulmonary artery is much lower than that in the aorta. It is now possible by direct catheterization of the right heart to take accurate measurements of the pulmonary blood pressure in man. The pulmonary systolic blood pressure in healthy people at rest lies between 18 and 30 mm. Hg. and the diastolic between 8 and 10 mm. Hg. With exercise the diastolic pressure, instead of rising, falls. This is due to a reflex dilatation of the pulmonary vessels. The systolic pressure remains unaltered (Riley *et al.*, 1948). This important reflex enables the right ventricle to increase its output in times of exertion with the least expenditure of energy.

There is a number of pathological conditions in which the right heart is exposed to abnormal strain, the commonest being those due to lesions of the heart, such as mitral stenosis or left ventricular failure (Thompson and White, 1936). This sequence has already been considered as part of the phenomenon of congestive heart failure. There is, however, another common sequence producing pulmonary heart disease or *cor pulmonale*, in which

the right heart alone is subjected to strain by reason of some lesion in the lungs or to an abnormality of the pulmonary circulation. Acute *cor pulmonale* occurs only as a result of obstruction of a major part of the pulmonary circulation by emboli, usually originating in the lower limbs. Multiple thromboses in small vessels may occasionally be widespread enough to have the same effect. In either case there is acute right heart failure which, if not rapidly fatal, terminates in recovery. Chronic *cor pulmonale* is a far more common condition than autopsy statistics suggest. In the vast majority of instances it is due to one of three diseases—chronic bronchitis and emphysema, pulmonary fibrosis, or kyphoscoliosis. Although the mechanism whereby chronic bronchitis and emphysema produce chronic right heart failure is still imperfectly understood, it can now be said with some confidence that the process is not a simple consequence of diminution in number of the pulmonary capillaries and increased resistance in the pulmonary circuit. This explanation can be excluded for three reasons : firstly, the operation of pneumonectomy, which removes half of the pulmonary circulation, does not give rise to pulmonary arterial hypertension (Brenner, 1935) ; secondly, there is no correlation between the degree of emphysema and the degree of right heart hypertrophy and failure. Severe chronic bronchitis and well-established hypertrophy of the right ventricular muscle are fairly frequently found to be associated with an insignificant degree of emphysema. Finally, it has recently been shown that far from there being a mechanical obstruction to the pulmonary circulation, the pulmonary blood flow is actually increased (Brooks, 1948 ; Riley *et al.*, 1948). When right heart failure develops in association with chronic bronchitis and emphysema, the cardiac output is greater than normal and the pulmonary blood pressure at rest during this " high output failure " is either normal or raised (Bloomfield *et al.*, 1946 ; Riley *et al.*, 1948). With exercise, however, the pulmonary pressure rises steeply, whereas under normal conditions it falls, suggesting failure or inhibition of the normal reaction to exertion which, by producing pulmonary vasodilatation, increases the output of the right ventricle. With the onset of right heart failure or an attack of bronchitis the pulmonary arterial pressure, even at rest, rises (Bloomfield *et al.*, 1946 ; Lenègre and Maurice, 1948).

The observations are confusing, but a unifying explanation has been offered by Motley *et al.* (1947), who showed that in healthy people a slight anoxæmia, induced by breathing an

atmosphere containing half the normal content of oxygen, produced a considerable rise of pulmonary blood pressure. This suggests that the pulmonary hypertension and high cardiac output are due to anoxia arising as a result of deficient oxygenation of the pulmonary blood in the emphysematous lung. Such a view is in keeping with the clinical observation that a comparatively mild bronchial infection in such cases will precipitate cardiac failure by causing obstruction to some of the bronchioles and further reducing the already inadequate oxygenation. This explanation can equally well be applied to pulmonary fibrosis and kypho-scoliosis. It does not follow that this is the only explanation. It is obvious from an examination of the lungs in emphysema or silicosis that there is a considerable degree of vascular obliteration and a reduction in the reserve of pulmonary vessels able to accommodate the increased blood-flow of physical exercise. Mechanical obstruction to the pulmonary circulation may therefore play some part in the cardiac failure of pulmonary heart disease, but in any case the basic factor must always be defective oxygenation, especially when the handicapped right heart is called upon to meet the need for increased oxygenation imposed by physical exertion.

To sum up, it appears that in pulmonary heart disease two factors are involved—failure of oxygenation and vascular obstruction. In emphysema the diminished tidal air and the deficient gaseous mixing within the enlarged alveolar spaces prevents a sufficient contact between oxygen and capillaries, and, in addition, the number of capillaries is diminished. In the presence of bronchitis or asthma the access of air to the alveoli may be impeded by bronchial secretions or spasm. In kypho-scoliosis the movements of the lung are impeded. In all these circumstances there is deficient oxygenation of blood which induces a reflex rise of pulmonary blood-pressure and increased cardiac output. In the case of emphysema and pulmonary fibrosis there is a significant destruction of alveolar capillaries, and in cases of pneumoconiosis there is frequently severe endarteritis with thrombosis in the smaller pulmonary arteries (Geever, 1947). These changes impede the increased blood-flow and add still further to the strain on the right heart.

Apart from the pulmonary diseases considered above, there is a small group of cases, described as primary pulmonary hypertension, in which the patients show gross elevation of the pulmonary blood-pressure and primary right heart failure. They

usually run a rapidly fatal course and show no evidence of anoxæmia. Such cases are rare, but the published reports suggest that they are all primarily vascular in origin and obstructive rather than anoxæmic. In some instances the ætiology has been established and the pulmonary arteries have been found to be occluded by tumour, parasites (bilharzia), thrombi or emboli. In others, pulmonary arterial disease has been found, but its nature not established. It seems likely that the majority of such cases are the result of some form of obstructive arterial disease, and there is at present no conclusive evidence of the existence of any disease of the pulmonary circuit comparable to primary or essential arterial hypertension which so commonly affects the systemic circulation.

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Rheumatic Carditis

The specific lesion of rheumatic fever is a small cellular nodule, hitherto believed to contain no demonstrable micro-organisms, and widely disseminated in what may be assumed to be susceptible or sensitized tissues ; these are the connective tissue framework of the heart, serous and synovial membranes, the subcutaneous tissue and the brain. The manifest conditions accompanying these lesions in these situations are respectively myocarditis and endocarditis, pericarditis and pleurisy, arthritis, rheumatic (skin) nodules and chorea.

These cellular nodules have the same essential structure wherever they occur. They have been most extensively studied in the myocardium, where they are known as Aschoff bodies. Strictly confined to the cardiac connective tissue, they occur most frequently in the posterior wall of the left ventricle near the base of the mitral valve and in the ventricular septum.

The morphology of the lesion changes during the course of

its evolution and its age can be estimated from the cytology (Gross and Ehrlich, 1934). Aschoff bodies can be found somewhere in the myocardium in nearly every case of active rheumatic fever, and when found they can be regarded as pathognomonic. Their value as a histological hall-mark has fostered the false belief that they are responsible for the functional failure of the myocardium in the acute phase of rheumatic carditis. It must be appreciated that they are always of insignificant size and are quite frequently difficult to find in cases of fatal acute cardiac dilatation. They always lie in the connective tissue and are clearly incapable of causing anything more than an insignificant degree of myocardial damage. It must also be borne in mind that occasional cases of fulminating and rapidly fatal rheumatic carditis are encountered in which Aschoff bodies cannot be found. Klinge (1933) suggested that the significant structural change in acute rheumatic carditis was extensive damage to the fine interstitial collagen framework which supports the muscle bundles, and proposed that rheumatism should be regarded as a disease of collagen. There is no doubt that the collagen of the heart is damaged in acute rheumatism. The earliest recognizable Aschoff body is characterized by a central focus of severely damaged collagen fibres which quickly becomes surrounded and then infiltrated by the typical mononuclear cells. In cases dying in acute febrile relapse and before any unequivocal Aschoff bodies can be identified, foci of altered collagen, often with a few lymphocytes, can usually be easily recognized. Gross and Ehrlich studied these changes and were satisfied that they occurred in acute rheumatism, but held that they were not sufficiently specific to be histologically diagnostic. The nature of this collagen change is not clear ; it is not a frankly necrotic process though often regarded as such. It appears to be a change in the chemical nature of the connective tissue ground substance that surrounds and binds together the fine collagen fibrils (Klemperer, 1950). It may be, therefore, that the fundamental structural change of acute rheumatism is a widespread alteration in the ground substance of the collagen fibres throughout the body, and that at points where this is of maximal severity it excites a secondary cellular reaction of histologically specific type.

Ætiology of Rheumatic Fever. The cause of rheumatic fever has been the subject of intensive research for many years, and, although finality has not yet been reached, the evidence now available gives valuable clues to both the ætiology and

pathogenesis. It may now be said that the disease is conditioned by recurrent faucial infection by Group A β-hæmolytic streptococci, and that altered sensitivity to some constituent of these streptococcal cells is the probable mechanism responsible for its manifestation.

The evidence incriminating the *Streptococcus pyogenes* is fourfold : the clinical association between streptococcal infections and rheumatic fever ; the finding of streptococcal antibodies in rheumatic fever patients ; the finding of streptococci in the heart in acute rheumatism ; and the experimental production of lesions resembling those of acute rheumatic carditis by repeated subcutaneous infections with Group A streptococci in animals.

The clinical association between acute tonsillitis or pharyngitis, due to *Streptococcus pyogenes*, and rheumatic fever has been well recognized for years. There is usually a history of repeated sore throats ; an attack or recrudescence of rheumatic fever frequently follows about a fortnight after a pharyngeal infection. This is an old clinical observation and has been fully confirmed (Collis, 1931 ; Sheldon, 1931), and Coburn and Pauli (1935) observed that epidemics of streptococcal throat infection among groups of patients with subacute rheumatism were frequently followed about a fortnight later by recrudescences of the rheumatism. Cultures taken from the surface of the tonsil in children suffering from active carditis and even during an attack of acute tonsillitis may contain no β-hæmolytic streptococci, whereas those taken from the depths of the tonsils are frequently positive. Furthermore, there is now little doubt that the long-continued prophylactic treatment of the rheumatic child by penicillin halts the progress of active carditis. That these associations are not due to coincidence is supported by the observation that the blood of rheumatic fever patients contains streptococcal antibodies to unusually high titres. The specific antibodies found include agglutinins, anti-hæmolysins, complement-fixing antibodies, precipitin (Coburn and Pauli, 1932), anti-fibrinolysin (Stuart-Harris, 1935), and an antibody to the M protein of Group A streptococci. Not only are antibodies to the *Streptococcus pyogenes* increased, but their production follows an abnormal course. In normal individuals a frank streptococcal infection causes a rapid but limited rise of antibodies, reaching a maximum in a few weeks. In rheumatic fever patients, however, the rise of antibodies is slow and persistent, gradually reaching high titres over a period of as long as six months (Coburn, 1936 ; Coburn and Pauli,

1939). Such findings strongly suggest that the streptococcal infection is not self-limited as it appears to be clinically, but persists in some hidden focus from which it continually releases antigens into the blood-stream.

In view of the evidence incriminating the *Streptococcus pyogenes* as the cause of rheumatic fever it is not surprising that numerous attempts have been made to culture streptococci from the actual lesions. In the majority of cases the attempt has failed, or when organisms have been obtained they have proved to be of questionable pathogenicity. The most significant work of this type was that of Green (1939), who, by employing a scrupulously careful technique, was able to cultivate *Streptococcus pyogenes* from the valves or pericardium in 8 out of 9 cases of rheumatic fever. In 5 of these, streptococci cultured from the throat during life proved to be of the same specific type as those isolated from the heart. This work has been confirmed by Collis (1939) and by Thomson and Innes (1940). It is highly significant that when streptococci have been isolated from the cardiac tissues they always present in very small numbers, and it must be emphasized that cultivation experiments such as these do no more than strongly suggest that acute rheumatic carditis is conditioned by β-hæmolytic streptococcal infection. Equal emphasis must be laid on the well-attested fact that the tissue reactions in acute rheumatism are totally dissimilar in almost every particular from those in which pyogenic streptococci multiply freely in the tissues of susceptible subjects.

The Allergic or Hypersensitive Mechanism. Klinge (1929–30 and 1933) considered that the peculiar alteration in collagen found in the lesions of acute rheumatism was in favour of regarding them as being due to the development of bacterial hypersensitivity. He therefore tried to reproduce the disease in rabbits by sensitizing them to a foreign protein (horse serum), followed by further injections of the same antigen. This work has since been repeated many times (Vaubel, 1932 ; Knepper and Waaler, 1934–35 ; Rich and Gregory, 1943 ; McKeown, 1947 ; Moore *et al.*, 1947 ; Ehrich *et al.*, 1949 ; More *et al.*, 1949). The antigen used has usually been a crude serum, but in one series of experiments purified fractions were employed (gamma globulin—More *et al.*, 1949). In all these experiments the proportion of animals showing a detectable lesion of collagen was disappointingly small. On the other hand, there was a general resemblance between the tissue response and those of acute

rheumatic infection, and in some experiments at least the resemblance has been close (McKeown, 1947 ; Biggart, 1949). What is perhaps a more valid criticism is that this treatment has also produced lesions which are quite different from those of rheumatism. In most cases the arteries show acute polyarteritis, and in some cases acute nephritis also occurred (More *et al.*, 1949). The only conclusion that can be drawn from this type of experiment is that there is sufficient resemblance between human rheumatic lesions and the experimental allergic lesions to suggest that rheumatism may be produced in a similar way by some different antigen.

Recently, Murphy and Swift (1949–50) have attempted to reproduce rheumatism in rabbits by following what they believe to be the usual course of events in man. They assumed that rheumatism in man followed repeated infections with Group A β-hæmolytic streptococci, but they argued that in all probability succeeding infections were caused by different serological types. They therefore induced repeated infections in rabbits by giving carefully graded small intra-dermal doses of Group A streptococci of different types which would produce infections well within the capacity of the animal to overcome. These infections were repeated at about monthly intervals over six months or a year. They noted that if they gave the injections at different sites the later ones produced a more violent local reaction than the early ones, suggesting the development of an increased sensitivity. After a variable number of injections some of their rabbits became ill with dyspnœa, leucocytosis, rapid irregular pulse and raised sedimentation rate, and died in four to fourteen days. In these rabbits they found endocardial and myocardial lesions which bear a striking histological and cytological resemblance to those of acute rheumatism (see Figs. 8/1 to 8/4). Furthermore, they did not find evidence of the polyarteritis which so often accompanies the cardiac lesions in animals made hypersensitive to foreign serum. On the other hand, arterial lesions were produced which closely resembled those of naturally-occurring rheumatic arteritis.

The interest of this work lies not only in the remarkable similarity of the lesions to those of human rheumatism, but in the fact that the mode of production is similar to that which is believed to operate in human cases. It is obviously hazardous to apply the results of animal experiment directly to man, but there now seems to be good evidence for the belief that acute

rheumatism is an expression of altered sensitivity to repeated infections by Group A streptococci of varying serological types. One question still remains unanswered. Recurrent Group A streptococcal tonsillitis is an excessively common experience at all ages, yet the proportion of those susceptible to this recurrent infection who develop recurrent carditis is inexplicably small. The same low incidence of cardiac lesions was found by Murphy and Swift in experimental animals.

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Other Forms of Carditis

Acute rheumatism and infective endocarditis account for the majority of cases of carditis. There are, however, three other

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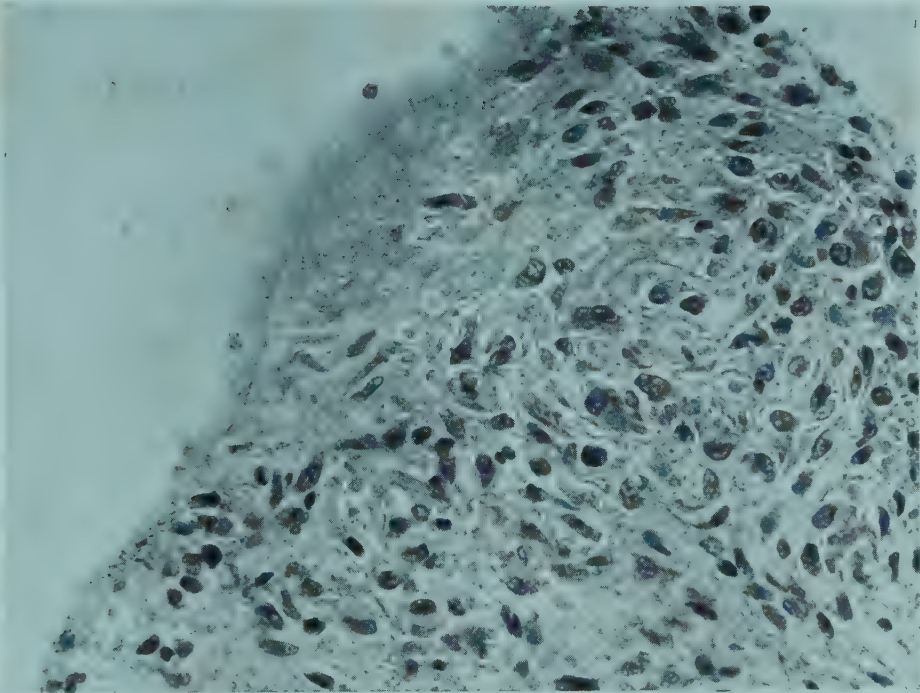
Fig. 8/1. Lesions of human rheumatic carditis and its experimental counterpart in rabbits. *Top :* From a ten years old boy who died after three months of active rheumatic fever. Nodule of large mono- and multi-nucleated cells among masses of swollen collagen at tip of trabecula carneæ. (Stained Masson trichome. × 388.) *Below :* Experimental lesion in same situation in the rabbit. (*Photographs kindly lent by Drs. Murphy and Swift.*)



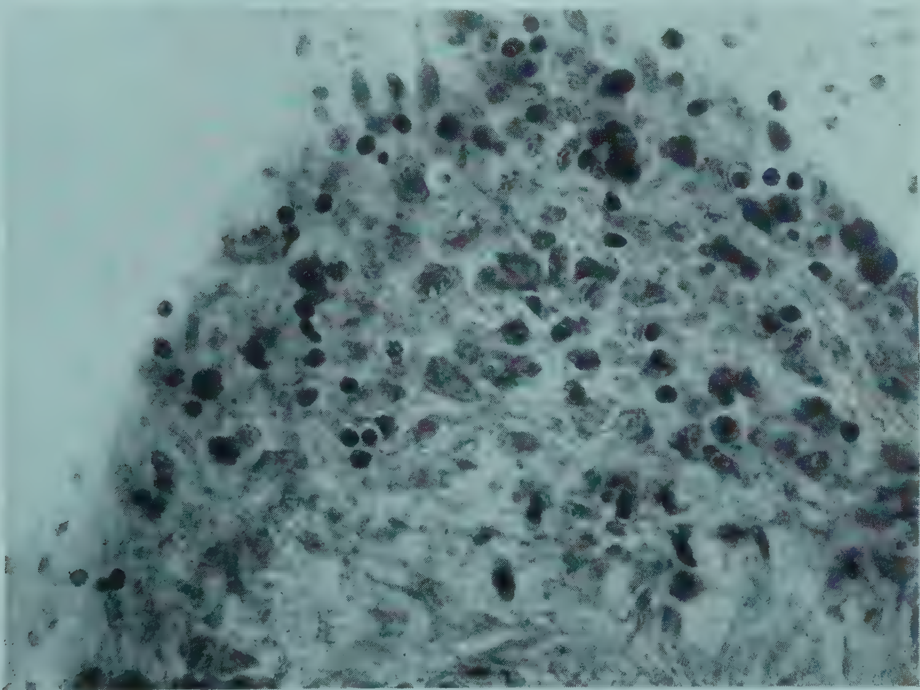


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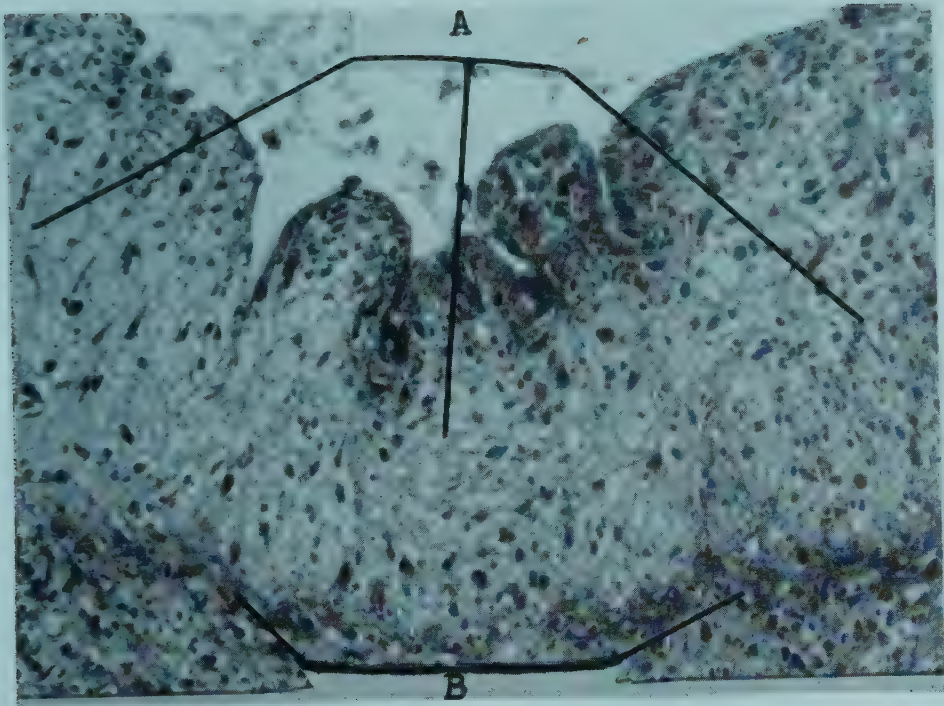
FIG. 8/2. Lesions in human rheumatic carditis and its experimental counterpart in rabbits.

Top : From the human heart referred to in Fig. 8/1. A—Polypoid palisading of endo- and subendocardial cells within network of swollen collagen in a mitral valve sulcus with necrosis of some of the most superficial cells. B—Fragmented internal elastic lamella. (Stained Weigert-hæmatoxylin and eosin. × 235.)

Below : Rabbit sacrificed fifteen days after the last of four infections the last of which was a superinfection. A—Polypoid palisading of endo- and subendocardial cells within network of swollen collagen in mitral sulcus. B—Fragmented internal elastic lamella. (Stained Weigert-hæmatoxylin and eosin. × 235.)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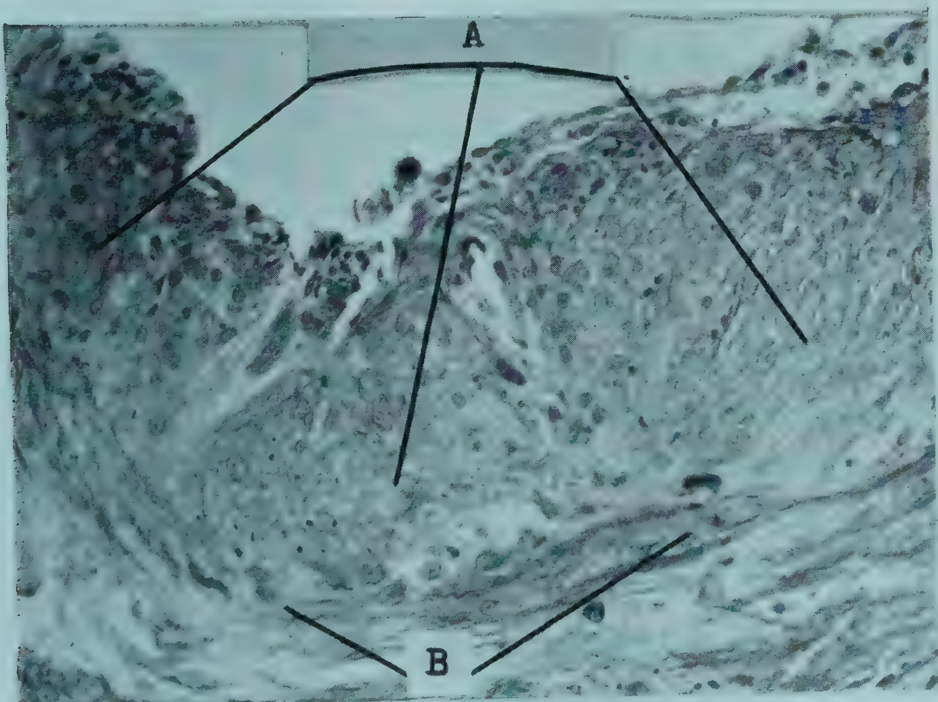
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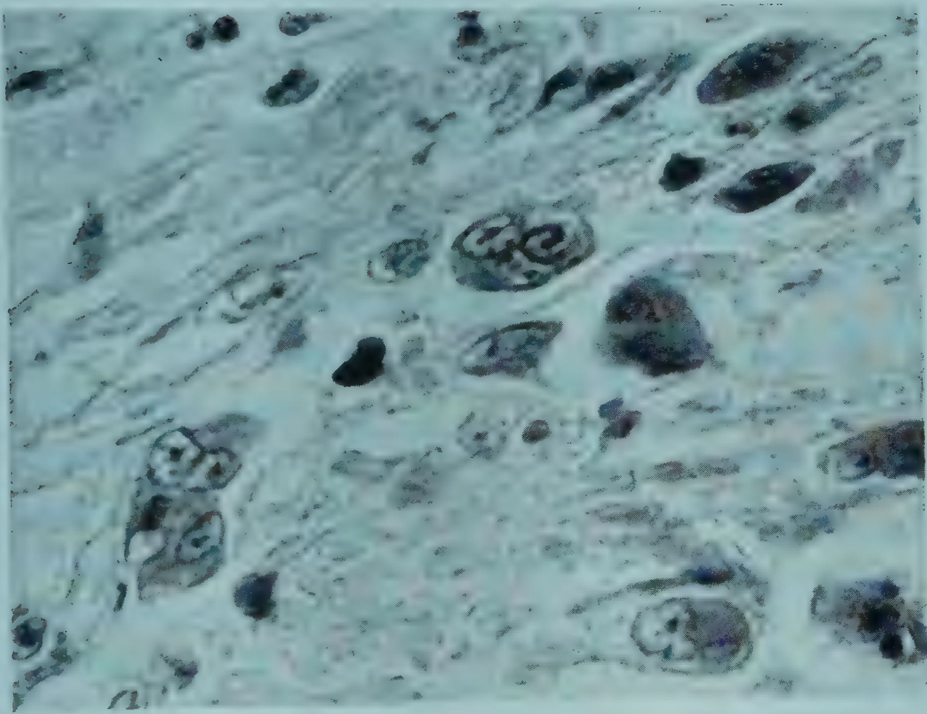
FIG. 8/3. *Top :* From the human heart referred to in Figs. 8/1 and 8/2. Dispersed among greatly altered collagen fibres in aortic sulcus are mono- and multinucleated granuloma cells characteristic of rheumatic fever. (Stained Weigert-hæmatoxylin and eosin. × 886.)

Below : Similar lesion in mitral sulcus of rabbit heart. (Stained hæmatoxylin and eosin. × 887.)



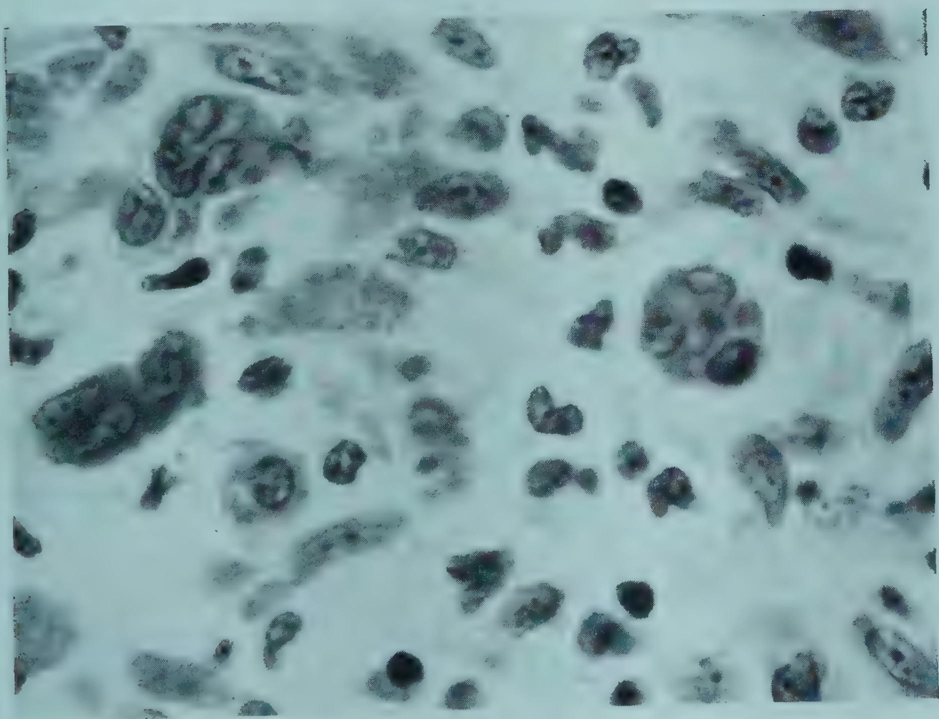


FIG. 8/4. *Top :* From right ventricle of human heart shown in previous figures. Diffuse reticular Aschoff body in myocardial interstitium ; many mononucleated and rare multinucleated granuloma cells dispersed in an interlacing network of swollen collagen that does not stain like fibrin with Masson trichrome method ; definite alteration of adjacent myofibres. Arteriolitis left lower corner. × 376.

Centre : Rabbit sacrificed while sick ten days following the last of six infections. Similar reticular granuloma in myocardial interstitium ; alteration of adjacent myofibres. (Stained Masson trichrome. × 399.)

Lower : Similar reticular granuloma in myocardial interstitium of human heart referred to above. Alteration of adjacent myofibres. (Stained Weigert-hæmatoxylin and eosin. × 420.)

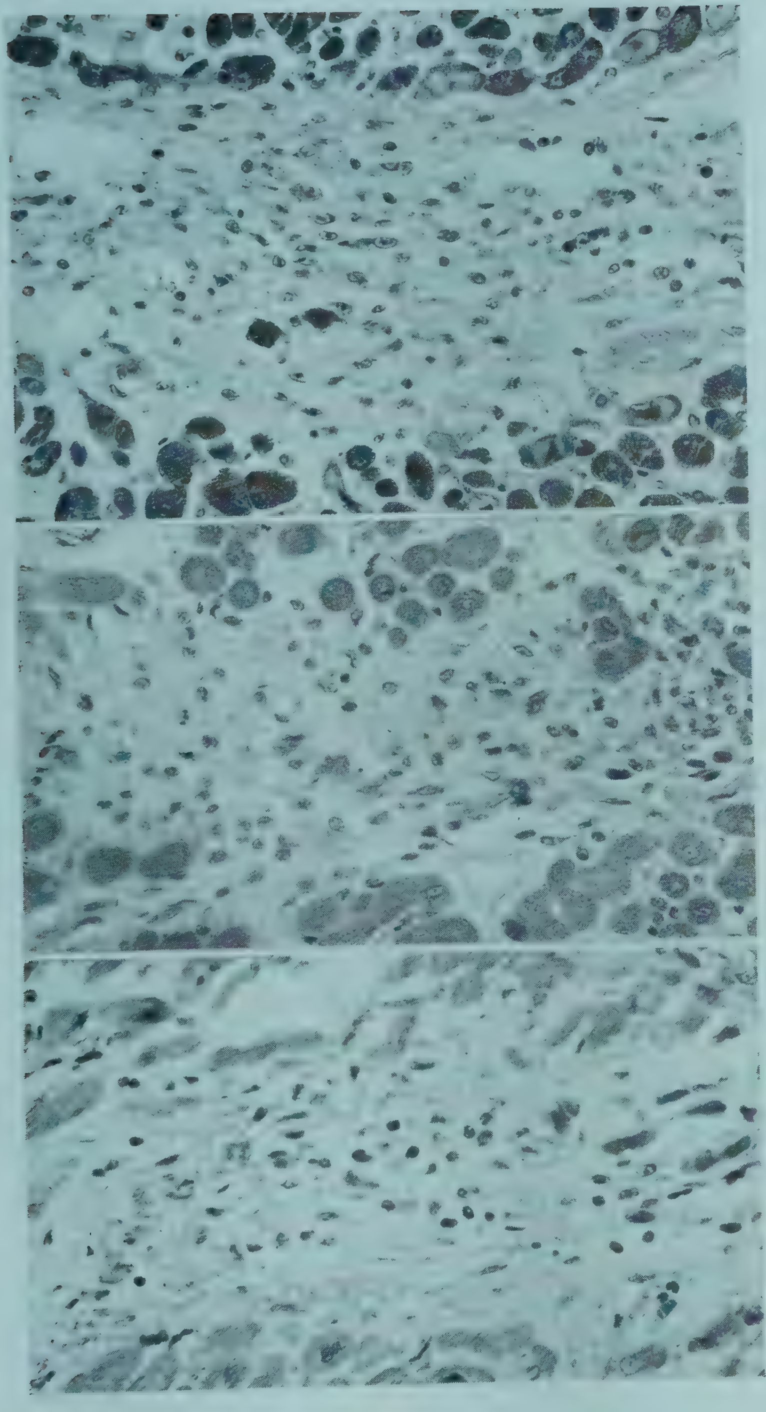


FIG. 8/5. Libman-Sacks endocarditis. *Top :* Mitral valve. Vegetations on anterior cusp (C) and posterior cusp (D). *Below :* Tricuspid valve. Vegetations on all three cusps (C, D, E) and on mural endocardium (F). Note pericarditis (G). (L. Gross. *Amer. J. Path.*, 1940, **16**, plate 78. *Reproduced by kind permission of the editor, Dr. Carl Weller.*)

varieties which, though uncommon, are of sufficient interest to warrant brief discussion. These are the endocarditis of Libman and Sacks ; the cardiac lesions of rheumatoid arthritis ; and a severe and usually fatal variety of myocarditis in which at present there is no clue pointing to any ætiological agent.

Libman-Sacks Endocarditis. In 1924, Libman and Sacks described four examples of a characteristic type of endocarditis, quite distinct from either rheumatism or infective endocarditis. In 1940, Gross described 27 similar cases and showed that the lesion was part of the picture of acute disseminated lupus erythematosus. In 1941, Klemperer and his colleagues showed that the lesion was not a true endocarditis, but a degenerative change in the sub-endothelial collagen of the heart and a part of a generalized disturbance of collagen throughout the body. The disease affects women almost exclusively (one of Libman and Sacks original cases was a male) and occurs in early adult life, with a mean age incidence of about twenty-five years and a duration of about six months. At some time during the illness the characteristic " butterfly " rash is usually present on the face. At autopsy endocardial lesions are visible macroscopically in about 40 per cent. of cases and microscopically in about 60 per cent. (Klemperer *et al.*, 1941). They occur as firm vegetations on the valves or mural endocardium and the mitral and tricuspid valves are most commonly affected and with about equal frequency. The aortic and pulmonary are less often affected, but again with similar frequency. In other words, the disease involves atrio-ventricular valves more than arterial valves, but without showing any predilection for the left side of the heart. The atrial or ventricular endocardium may be affected quite apart from the valves and the pockets beneath the atrio-ventricular valves are a frequent site of involvement. The vegetations may take either of three forms (Gross, 1940)—lines of nodules along the valves, resembling those of rheumatic endocarditis but rather larger ; flat, warty plaques ; or large exuberant masses (Fig. 8/5). In some cases infective endocarditis may be superimposed (Klemperer *et al.*, 1941).

Microscopically the essential change is hyaline swelling of the sub-endothelial collagen. This projects or erupts through the endocardium and also acts as a foreign body, causing a secondary inflammatory response characterized by macrophages and plasma cells and new capillaries. Later new fibrous tissue formation follows the inflammatory response and may in turn undergo

hyaline change. One of the characteristic microscopical features of these lesions is the presence of small rounded hæmatoxylin staining bodies which Klemperer and his colleagues (1950) have shown to be derived from the nuclei of the mesenchymal cells. These bodies are not found exclusively in the heart, but occur in all the lesions of lupus erythematosus.

Apart from the endocardium, characteristic changes occur in the collagen of the valve rings and pericardium. The myocardium is not affected, though there may be small foci of collagen change in the interstitial tissue. These, however, never resemble Aschoff bodies, though in this connection it is possibly significant that Gross found that 5 of his 27 cases were complicated by acute rheumatic infection.

Although the cardiac changes in disseminated lupus erythematosus are striking and characteristic, it is essential to realize that they are only a local expression of a generalized disorder of collagen which can be seen equally well in the sub-endothelial layers of serous membranes, in small arteries, or in the characteristic " wire-loop " swelling of the interstitial connective tissue of the glomerular tuft. The cause of the disease is quite unknown. Klemperer and his co-workers (1942) drew attention to the morphological similarity of the collagen change in disseminated lupus, rheumatic fever, rheumatoid arthritis and polyarteritis, and suggested that they might be grouped together, at least for descriptive purposes, as " collagen diseases " because they believed that this peculiar hyaline swelling of collagen was the fundamental lesion in all of them. They did not suggest that they had a common ætiology. Other workers have accepted this view and have gone further in postulating a common allergic background (Bergstrand, 1946 ; Teilum, 1948 ; Aegerter and Long, 1949). In favour of this view are the two observations that collagen damage can be induced experimentally by sensitization to foreign protein and that all these so-called collagen diseases respond to cortisone or A.C.T.H. On the other hand, morphologically identical collagen damage can be produced by other non-allergic agencies and, as Klemperer himself has pointed out (1950), there is not enough available evidence to justify any final conclusion with regard to the ætiology of this group of diseases.

Cardiac Lesions in Rheumatoid Arthritis. Rheumatoid arthritis *per se* is not a fatal disease ; its victims usually die of intercurrent disease. Some of them will obviously die of rheumatic heart disease, and this number is much greater than would be

expected. Bayles (1943) found at autopsy that 6 out of 23 cases of rheumatoid arthritis had rheumatic heart disease ; in one of these the lesions were still active. Young and Schwedel (1944) reported a series of 38 autopsies in 24 of which they found signs of rheumatic heart disease. In 2 cases there was active valvulitis and in 1 they found Aschoff bodies. Rosenberg, Baggenstoss and Hench, in the same year, reported a series of 30 autopsies, 16 of which had rheumatic heart disease and in 7 it was the cause of death. Thus, in an aggregate of 111 autopsies on cases of rheumatoid arthritis, there were 46 with rheumatic heart disease, an incidence that suggests some close association between the two diseases. It should, perhaps, be mentioned that in all these investigations only cases of clinically classical rheumatoid arthritis were included in the published series, and the cardiac lesions were indistinguishable from those of acute rheumatic carditis. In a separate report, however, in which Baggenstoss and Rosenberg (1944) describe two hearts in detail, some doubt is cast on the rheumatic origin of the heart lesions. In both cases the lesions, though superficially like those of active rheumatism, were characterized by unduly large foci of collagen necrosis, and they noted that such granulomata resemble the subcutaneous rheumatoid nodule more than the true rheumatic lesion. Bywaters (1950) came to a similar conclusion. He found 8 examples of myocarditis amongst 27 cases of rheumatoid arthritis, and of these he regarded one as a rheumatoid lesion of the heart and two others as probably so. From these reports it seems fairly certain that valvular and myocardial lesions closely resembling those of rheumatism occur with remarkable frequency in cases of rheumatoid arthritis and are the cause of death in a significant proportion. What is not clear is whether the lesions are truly rheumatic and thus indicate a high degree of coincidence between the two diseases. or whether the cardiac lesions are another manifestation of the rheumatoid process in the connective tissue stroma of the heart. There is obviously a need for further careful investigation of the whole of the morbid anatomy of rheumatoid arthritis.

Primary, Isolated, or Fiedler's Myocarditis. These three terms are used to denote a severe, usually fatal, myocarditis of unknown ætiology unassociated with any condition known to cause demonstrable damage to the myocardium. The number of diseases associated with lesions in the heart muscle is considerable and minor grades of myocarditis are common. The more important of these have already been discussed, but there still

remains a large and heterogeneous group that can be conveniently considered under three headings :

Toxic myocarditis :
Diphtheria
Typhoid

Direct invasion of the heart muscle by
i. Micro-organisms
ii. Rickettsia
iii. Parasites

Myocarditis associated with
Non-specific infections
Friedreich's ataxia

In the first group the heart muscle suffers toxic damage, but there is no structural alteration of any significance. Small isolated groups of muscle cells are found to be necrotic and to be surrounded by localized cellular infiltration. These foci afford a valuable diagnostic index, but do not throw any light on the nature of the functional failure of the heart muscle.

Bacterial infection of the heart occurs as a part of a generalized septicæmia, even in the absence of infective endocarditis. Organisms settle in the heart muscle and produce small foci of acute inflammation which may progress to abscess formation if the patient survives long enough. Since the advent of effective therapy these cases have become increasingly rare.

In Rickettsial diseases, typified by typhus fever, the heart is involved with many other organs and small lesions occur in the vicinity of the small vessels. Of the larger parasites two are of particular importance. The South African trypanosome (*T. Cruzi*) regularly infests the cardiac muscle fibres. Localized, tightly-packed collections of Leishman-Donovan bodies can be seen surrounded by an infiltration of macrophages, lymphocytes and plasma cells. These lesions are numerous and cause sufficient damage to lead to fatal cardiac failure. The other parasite, which is of more practical importance in Europe is *Trichina spiralis*. The larval forms of this worm pass from the intestine *via* the blood-stream to the skeletal muscle and heart. In the former they encyst and can readily be recognized, but in the heart they rapidly die and become unrecognizable. The heart may show a severe myocarditis with diffuse interstitial infiltration and foci of muscle necrosis surrounded by a denser cell reaction.

The cells are mainly eosinophils and neutrophil leucocytes, but lymphocytes and macrophages are also present. It is worth noting that even if larvæ are not demonstrable in the heart, they can readily be found in skeletal muscles, especially the diaphragm.

Apart from the specific diseases considered so far, myocarditis may be encountered in many, apparently non-specific, infections. Gore and Saphir (1947) in particular have studied this subject and have been able to find myocarditis in the hearts of patients dying of a bewildering variety of infective diseases. In the vast majority of cases the carditis is an incidental finding and of no clinical importance, but they quote a significant number of cases of apparent cardiac death associated with microscopically demonstrable myocarditis occurring in patients with respiratory infection of various kinds.

An interesting example of myocarditis is that reported by Russell (1946) in 4 cases of Friedreich's ataxia, all of whom died of cardiac failure and at autopsy had hypertrophied hearts. Microscopically the hearts showed increased interstitial fibrous tissue and cellular infiltration ; in 1 case there was active muscle destruction. Similar findings have been reported in a single autopsy case (Hejtmancik *et al.*, 1949). In view of the comparative rarity of Friedreich's ataxia, it is improbable that the association with myocarditis could be fortuitous, though at present we have no clue to any common ætiology.

In all the examples of myocarditis briefly mentioned above, the heart has been the site of some recognized disease, or the cardiac lesion clearly associated with some disease process in another part of the body. There still remains a variety of myocarditis which appears to be unassociated with any known disease. Such cases have been called primary, isolated or Fiedler's myocarditis. This disease occurs at any time from infancy (House, 1948 ; Raeburn, 1948) to old age (Saphir, 1942 ; Marcuse, 1947). It may run a rapid course of a few days or weeks, or a long course over months. The patient may die suddenly or more slowly in congestive failure. At autopsy the heart is dilated and is nearly always overweight, even in cases of short clinical duration. As a rule, there is little to be seen by the naked eye ; the myocardium is flabby and perhaps pale, and occasionally there may be focal points of softening or discoloration. In other organs the only findings are those of cardiac failure and perhaps terminal respiratory infection. Microscopically there is either a diffuse infiltration of the interstitial connective tissue of the

heart by inflammatory cells, or focal granulomatous lesions which cause destruction of the muscle (Fig. 8/6). The former lesion is much more common, but this may be due to the fact that being diffuse it can be recognized in random sections, whereas the focal type will be more easily missed. In the diffuse interstitial type of myocarditis the cellular infiltration varies. It is usually composed of lymphocytes and plasma cells, but in a few cases it is mainly eosinophilic (Reinhart, 1946). As a rule, the muscle fibres are little affected and the lesion remains predominantly interstitial. The localized granulomatous lesion is characterized by focal areas of cellular infiltration associated with considerable local muscle destruction (Magner, 1939 ; Covey, 1942), and the cellular infiltration contains lymphocytes, macrophages, plasma cells and sometimes polymorphonuclears. From the published descriptions it appears that this ill-defined variety of myocarditis has neither a fixed clinical pattern nor a specific pathological lesion, and nothing is at present known of its ætiology.

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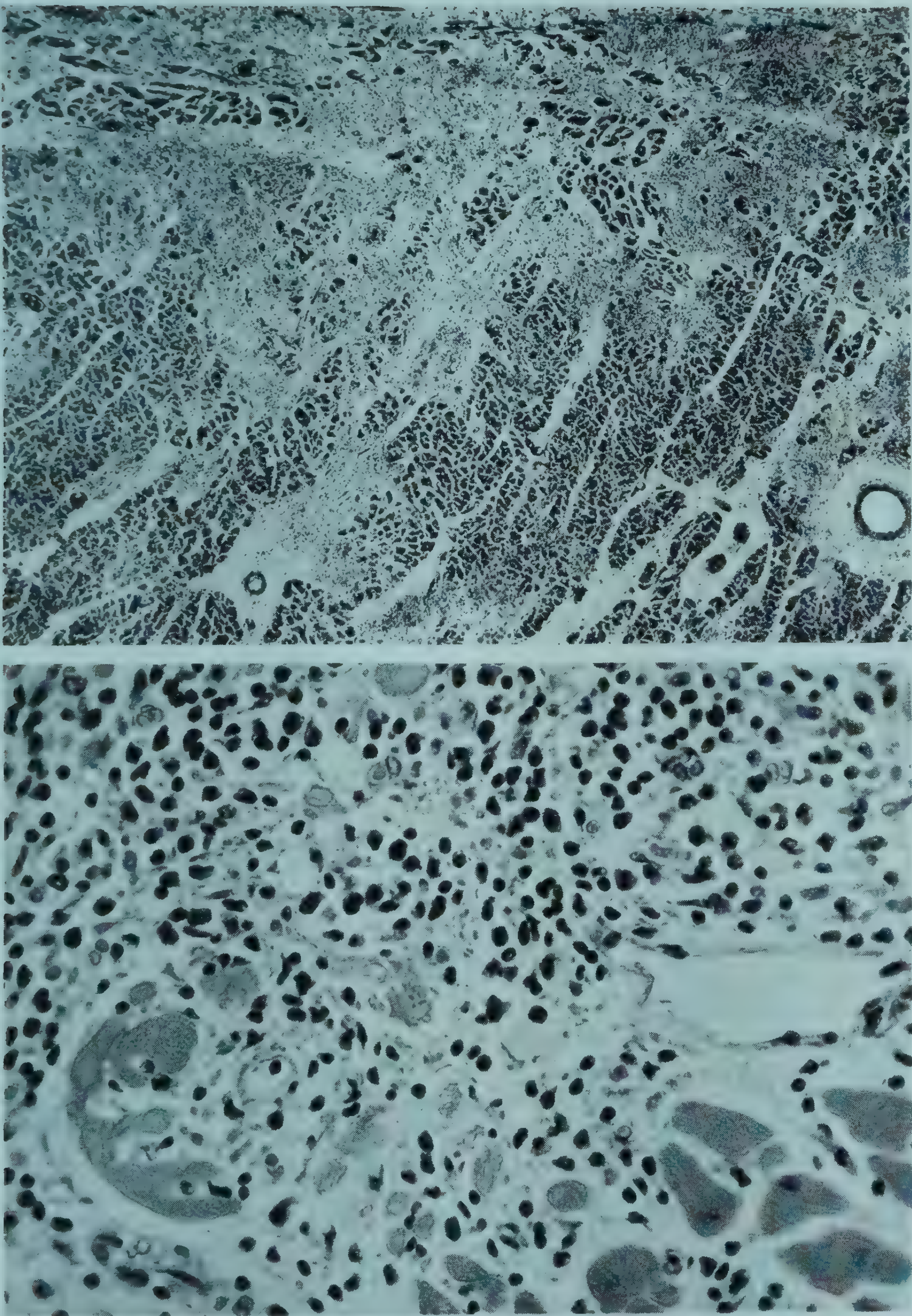

FIG. 8.6. Isolated (Fiedler's) Myocarditis. *Above :* Right ventricle showing widespread focal myocarditis. × 50. *Below :* High power view showing destruction of muscle and mixed cellular infiltration. × 460.

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THE BLOOD VESSELS

Primary Arteritis

Apart from a variety of inflammatory diseases in which arteries are incidentally involved, there are three diseases in which acute arteritis is the essential or only lesion. These are polyarteritis nodosa, giant-cell arteritis and thromboangeitis obliterans.

Polyarteritis nodosa is a disease of striking variability, affecting individuals of any age and either sex, but commonest in males in the fourth decade. Its clinical course is usually febrile and may be short and stormy with death in a few weeks or, more often, after several months. Its course is punctuated by a series of apparently unrelated and unpredictable incidents, due to involvement of the arteries of various organs. Clinical diagnosis is invariably difficult and many cases are first recognized at autopsy. This accounts for the common misconception that polyarteritis nodosa is always fatal. An increasing number of recoveries is being recorded ; Klein (1949) reports 2 histologically proven recoveries and quotes 9 other published cases, whilst Harris and his co-workers (1939) estimate that 10 per cent. of cases recover.

Nodular aneurysmal swellings along the course of the small arteries, usually just before they enter the viscera, constitute the classical anatomical hall-mark of the disease. These nodular swellings are frequently absent, the small arteries affected being visible as opaque white cords. Sometimes, however, both these changes are absent and diagnosis depends upon the histological picture. The distribution of the lesions between various arteries is also variable, but the vessels of the kidney, heart, liver, spleen, lungs, small intestine and spermatic cord are amongst those more often involved. Polyarteritis nodosa is essentially a disease of the smaller muscular arteries, so that the lesions are seen either in arteries within viscera or in those just entering them.

Microscopically the essential lesion is necrotizing panarteritis, commencing as medial necrosis and usually involving only part of its circumference. The necrotic areas are acidophilic and give a positive stain for fibrin. As a rule, the internal elastic membrane is fragmented, or even totally destroyed over the affected area. Accompanying the necrosis there is a cellular infiltration of the whole arterial wall, together with the adjacent connective tissue immediately outside the adventitia. Polymorphonuclears and macrophages are always seen ; there may be a heavy infiltration

of eosinophils, but this varies in incidence and intensity. The walls of the affected segment of the vessel may become so weakened that it dilates to form a local aneurysm ; with or without such dilatation there may be thrombosis, or the intima may undergo active fibroblastic proliferation, producing reduction or obliteration of the lumen. In any case, the circulation is interrupted and infarction is a very common sequel.

Ætiology. Recent investigations suggest that hypertension and hypersensitivity are involved in the ætiology of polyarteritis nodosa. The fibrinoid necrosis of the vessel wall is essentially similar to that seen in rheumatic fever, and Klemperer has included polyarteritis nodosa in the group of collagen diseases. It occurs in association with rheumatic fever and asthma too frequently for the association to be fortuitous (Bergstrand, 1946, 1950). Metz (1931) produced acute arteritis in animals previously sensitized to a foreign serum by the intravenous injection of the same antigen. A similar lesion was produced, using hæmolytic streptococci as the allergen. Knepper and Waaler (1935), unable to produce arteritis by foreign sera, were able to do so in animals compelled to take forcible exercise in a treadmill, a result which suggests that mechanical strain on the vessel wall might be an additional factor. Since then serum arteritis has been produced by Rich and Gregory (1943) and numerous other workers. One of the criticisms of experiments of this kind is that, in addition to acute arteritis, some of the animals showed lesions resembling rheumatic nodules or the lesions of acute glomerulitis. Since, however, arteritis in man may also occur in the presence of rheumatism, and since Davson and others (1948, 1950) have shown that an " explosive " glomerulitis is a frequent accompaniment of human polyarteritis, this criticism is hardly valid. These experiments having drawn attention to the possible allergic basis of polyarteritis, cases have been described in man in which polyarteritis has followed an allergic reaction to a known substance (Rich, 1945 ; Berblinger, 1950).

The other way in which polyarteritis has been produced experimentally is that of Smith and Zeek and their colleagues (1944, 1947, 1948), who wrapped silk cloth around the kidneys of rats and dogs. This caused a severe prolonged hypertension, and in about half of the animals lesions of polyarteritis were present in various systemic vessels, but not in the pulmonary vessels. These workers failed to produce polyarteritis by serum hypersensitivity and concluded that arterial hypertension was of major significance.

It should be noted, however, that infection of the operation site was a frequent occurrence in their animals, and though they could not find any difference in the incidence of arteritis between infected and uninfected animals, Kipkie (1950), who repeated their experiments with and without deliberate infection by staphylococci, found arteritis only in the infected group. The significance of these experiments for human polyarteritis is not clear, though it is very unlikely that hypertension alone is the cause. Necrosis of the vascular walls certainly occurs in malignant hypertension, but the structural changes differ significantly from those of polyarteritis. Nevertheless, arterial hypertension is a very frequent accompaniment of human polyarteritis, and, taken in conjunction with the fact that Knepper and Waaler could produce serum polyarteritis only in those animals which were forcibly exercised, it may well be that strain on the vascular wall is a precipitating factor in the causation of polyarteritis.

To summarize, there is good evidence that both hypersensitivity and hypertension are factors in the production of polyarteritis in experimental animals and in human cases, though in the latter neither can be constantly demonstrated.

Giant-cell or Temporal Arteritis. The individuality of this disease was first recognized in 1934 by Horton, Magath and Brown, though isolated case reports had appeared previously. Since that time about 120 cases have been recorded and many more have been recognized. Giant-cell arteritis affects either sex but shows a strikingly late age incidence, the vast majority of patients being over sixty. Like polyarteritis, it produces a febrile illness, but this is less severe. The striking clinical symptom is persistent, and severe pain in the head, which usually precedes the appearance of thickened, tender and nodular temporal arteries. The temporal arteries are most often involved, but it is usual to find some signs of involvement of other cranial vessels, such as the occipital arteries, and in the limited number of cases that have come to autopsy, widespread involvement of other deeper arteries has been observed. The vast majority of patients recover after an illness of six months or more, but about 20 per cent. suffer permanent blindness of one or both eyes from vascular involvement.

The vessels most often involved are the temporal, occipital and facial arteries, but in cases observed at autopsy the aorta, innominate, carotid, sub-clavian, radial, coronary and femoral arteries have been involved. The disease is, therefore, widespread and affects vessels of a larger size than does polyarteritis nodosa.

There are no characteristic naked-eye changes. In large vessels, such as the aorta or carotid arteries, there may be some degree of local dilatation and a thin layer of thrombus over the affected part, but this is not constant and the lesion may be readily overlooked, especially in vessels already atheromatous. In smaller vessels like the temporal arteries the wall is thickened, the lumen is reduced and may be occluded by thrombus. The histology is characteristic, but the lesions are unevenly distributed, so that sections at several levels may be necessary to disclose a typical area. The lumen in the case of small arteries is greatly reduced and in about a quarter of the cases is occupied by a thrombus. The intima is greatly thickened and shows two layers, the inner consisting of newly-formed connective tissue with active fibroblasts lying in a mucoid background, the outer being heavily infiltrated by inflammatory cells. Lymphocytes, macrophages, plasma cells and polymorphonuclears are usually present, but eosinophils are rare. At the junction of intima and media there is a variable number of multinucleate giant cells, usually irregular in size and shape. This constitutes the most constant and characteristic feature of the lesion. The internal elastic lamina is fragmented and considerable parts of it may have disappeared (see Fig. 8/7).

Ætiology. Nothing is known of the ætiology of giant-cell arteritis. The nature of the lesion does not suggest a direct infection and no micro-organism has been isolated. In a significant number of cases the disease has followed some form of local infection, and this, together with its resemblance to polyarteritis, has naturally suggested an allergic pathogenesis, but there is no reliable supporting evidence to justify any final conclusion.

Thrombo-angeitis Obliterans, or Buerger's Disease. This is an uncommon disease occurring in males in the first half of adult life. Its distribution is peculiar in that it is virtually limited to the vessels of the distal halves of the extremities, though it may affect the thigh and upper arm vessels. Both artery and vein are involved and the superficial veins, apart from the deep vessels, may be affected. The vascular occlusion leads to ischæmia of the limb, frequently ending in gangrene. Nearly all the material available for study is derived from amputated limbs, and since amputation is not undertaken until gangrene or intolerable pain make it unavoidable, the majority of specimens show only the late scarred stages of the disease,

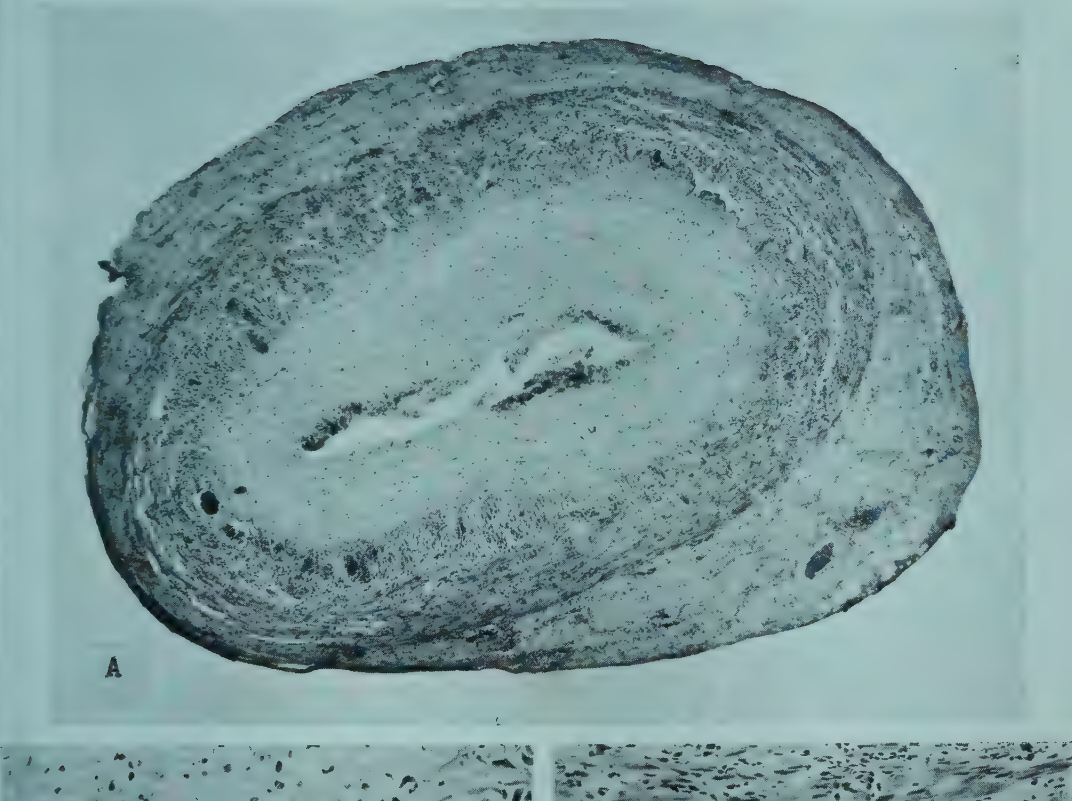

FIG. 8/7. Temporal (Giant cell) Arteritis. A—Temporal artery showing trace of thrombus thickened intima, cellular infiltration of media and fibrosis of adventitia. (× 40). B—Junction of intima and media showing fragmentation of elastica, giant cells and cellular infiltration. (× 190). C—Adventitia showing fibrosis with involvement of nerves and slight cellular infiltration. (× 120).

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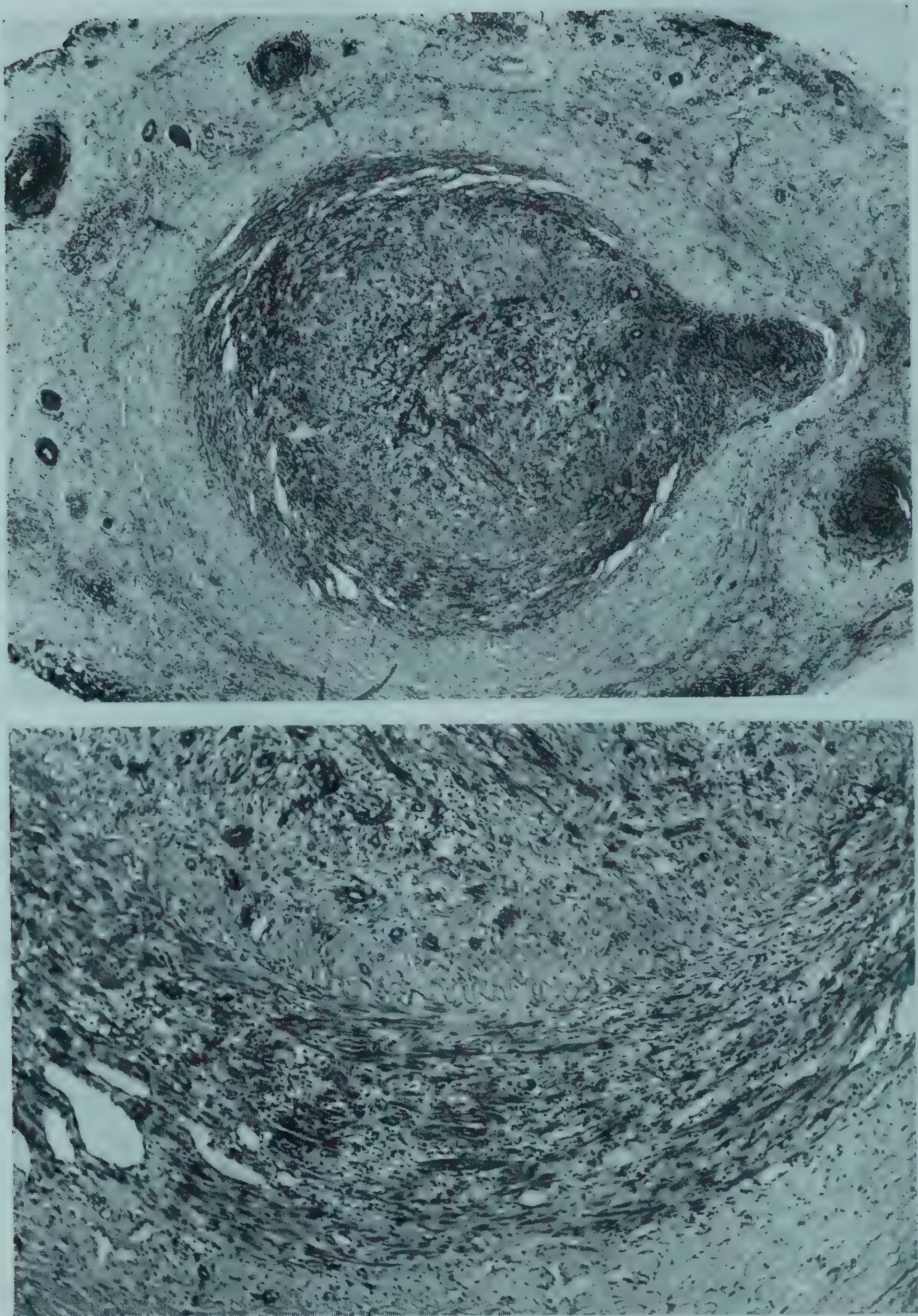

FIG. 8/8. Thrombo-angeitis obliterans. Transverse section of artery from male æt. twenty-five years. *Above :* The lumen is occluded by organizing thrombus. The media shows penetration by many large vasa vasorum. The adventitia is fibrosed. (Stained hæmatoxylin and eosin. × 33.) *Below :* Field showing organization of thrombus, intact internal elastic lamina, cellular infiltration and vascularity of media without any necrosis. (Stained hæmatoxylin and eosin. × 95.)

giving little indication of the intensity of the inflammatory process during the acute stage.

Histology. The acute stage of thrombo-angeitis obliterans is characterized by an active inflammatory infiltration of artery, vein and adjacent connective tissue. The walls of both arteries and veins are infiltrated by macrophages, polymorphonuclears and lymphocytes, the exudate lying in the interstices between the muscle fibres of the media and spreading out beyond the adventitia to involve the loose connective tissue surrounding the vessels and nerves. It is of interest to note that the inflammatory infiltration is diffuse around the whole circumference and is not associated with necrosis of the media or destruction of the elastica (Fig. 8/8). There is consequently none of the destruction of the vessel wall that is seen in polyarteritis. This acute angeitis is associated from its earliest stage with thrombosis, and the thrombus is infiltrated with inflammatory cells just as severely as the vessel wall. As the lesion progresses, new vessels grow in from the vasa vasorum and organize the clot, so at this stage the media is traversed by many new vessels (Fig. 8/8). During this stage of early organization there are often small circumscribed foci of cellular infiltration in the clot. At first these contain polymorphonuclears, which later are replaced by large macrophages and multinucleate giant cells. Since the latter are seen only in the organizing clot, they are clearly distinguishable from those of giant-cell arteritis which lie at the junction of media and intima. The intima in thrombo-angeitis does not undergo proliferation, probably because the lumen is occupied by clot from the earliest stages. As the lesion heals, the cellular infiltration becomes almost wholly lymphocytic and gradually disappears. The clot is removed and replaced by a rather loose connective tissue containing macrophages filled with hæmosiderin and new vessels. The latter often enlarge and develop well-defined medial coats of muscle and elastic tissue. As the clot organizes and shrinks the media around it contracts, causing the muscle to appear thick and the elastica to be thrown into exaggerated folds. The final picture, most often seen in amputated limbs, is that of an artery with accompanying veins and nerves bound together in dense fibrous tissue. The media is intact and contracted around a loose fibrous core traversed by several well-formed vessels. The latter run irregularly and do not re-establish the lumen.

The ætiology of thrombo-angeitis is unknown. It affects males of all races (it was an accident that most of Buerger's original

cases were Jews), and seems to be associated with heavy cigarette smoking. The essential lesion is an acute inflammatory one without any collagen necrosis, in this respect differing from polyarteritis. Attempts to culture micro-organisms have been unsuccessful.

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Arterial Degeneration

Degenerative arterial disease—arterio-sclerosis—is an inevitable accompaniment of the natural process of ageing and it is rare to see an autopsy on a patient over sixty in whom there is not clear evidence of vascular deterioration. On the other hand, it must be remembered that severe degrees of vascular sclerosis are consistent with full physical activity. It is, therefore, pertinent to consider the various types of arterio-sclerosis and ascertain which of them cause symptoms and how they do so.

The term " arterio-sclerosis " embraces three morphologically distinct lesions—diffuse hyperplastic sclerosis, medial sclerosis, and athero-sclerosis. Each variety has its own individual anatomical incidence in the arterial tree and its own ætiological background and clinical consequences.

Diffuse hyperplastic sclerosis is a disease of the small muscular walled visceral arteries in which the intima is thickened by alternating layers of fibrous and elastic tissue. The medial muscle may be hypertrophied or thinned. This lesion affects the kidney vessels most severely, the spleen and pancreas less severely, and other arteries to minor degrees. In the absence of hyper-

tension it occurs late in life and the intimal thickening is usually associated with thinning of the medial muscle. More often it is associated with hypertension and then there is often medial muscular hypertrophy and intimal thickening, especially if the patient is young. It is probable that the intimal overgrowth is a reaction to the stress of internal pressure and a compensatory phenomenon. This lesion is quite distinct from the hyaline degeneration of the arterioles which also occurs in hypertension and the arteriolar necrosis of malignant hypertension. There is no convincing evidence that diffuse hyperplastic sclerosis causes any narrowing of the lumen during life, though the contracted appearance of fixed vessels in histological sections often suggests this, nor is there evidence that it causes ischæmia of the tissues.

Medial sclerosis is a disease of curiously limited distribution, affecting the larger arteries of the lower limbs almost exclusively. It takes the form of isolated rings of calcification in the media so that the affected vessel looks and feels like a miniature trachea. It is essentially a disease of old age and is not associated with hypertension. Microscopically the earliest change consists of a fine dust-like deposit of calcium salts in the interstitial connective tissue between the muscle fibres or around the elastic fibres. These deposits enlarge and coalesce to form firm rings of calcification with an accompanying loss of the intervening muscle. The lesion is wholly medial and does not encroach on the intima or cause any narrowing of the lumen. It is important in this respect to make a clear distinction between Mönckeberg sclerosis in the media and secondary calcification in an overlying focus of athero-sclerosis ; the latter may cause severe narrowing of the lumen.

Athero-sclerosis is a focal disease that affects the intima of all the larger arteries from the aorta down to the larger visceral vessels. It is closely associated with age and occurs in the absence of hypertension but tends to appear earlier and to be more severe in the presence of hypertension. It takes the form of plaques of intimal thickening composed of fibrous tissue overlying a mass of fatty material. In early lesions the fat, which consists largely of cholesterol esters, lies within large foamy macrophages, in older lesions the fat often lies loose in the tissues as a mass of acellular *débris*. Calcium salts are liable to be deposited in the latter. Of the three forms of arterial degeneration, athero-sclerosis is the only one that causes disability or death and it does so by encroaching on the lumen and causing ischæmia of essential tissues.

Although athero-sclerosis is a widespread disease and affects the larger arteries throughout the body it only produces significant ischæmia in three sites : the brain, the heart and the lower limbs, suggesting that in these sites there is some additional factor which is not operative elsewhere. In the cerebral arteries this factor is clearly thrombosis and since the brain is peculiarly sensitive to lack of oxygen even comparatively minor thrombi show their presence immediately. At autopsy on patients who have suffered from cerebral softening the affected vessels can usually be found and the lesion can be recognized as thrombosis, either recent or organizing. In sites where thrombosis has not occurred the cerebral vessels may be severely atheromatous but they are not narrowed ; they are, in fact, often irregularly dilated.

In the coronary arteries quite a different state of affairs is usually seen. At autopsy in patients who have suffered from myocardial ischæmia the coronary arteries usually show a variety of lesions. Ordinary localized intimal plaques of fatty atheroma can nearly always be seen but it is doubtful whether these cause ischæmia. If death has followed soon after an attack of myocardial infarction, a recognizable focus of thrombosis can usually be found though detailed dissection may be necessary. The outstanding lesion, however, in the majority of cases is the conversion of parts of the main coronary trunks into rigid tubes with pin-hole lumina. These are ordinarily regarded as athero-sclerotic foci but Harrison and Wood (1949) who studied these lesions by opaque injection and clearing, and subsequently histologically, decided that many of them were certainly healed thrombi and that in the rest thrombosis could not be excluded. Duguid (1946, 1948) has similarly studied these lesions by frozen sections and believes that they are all thrombotic in origin. Alternatively, it has been suggested (Winternitz *et al.*, 1938) that hæmorrhage occurs into the depth of athero-sclerotic plaques and distends them. This will cause narrowing of the lumen and very frequently, by breaking through the internal surface, lead to thrombosis within the vessel. There is no doubt that this occurs, but it is doubtful whether it accounts for more than a small proportion of narrowed coronary arteries. From our own observation we believe that the factor which differentiates the occlusive variety of coronary athero-sclerosis from non-occlusive athero-sclerosis in other sites is *thrombosis with subsequent organization.*

The third site at which athero-sclerosis causes ischæmia is the lower limb. Here the disease has not received such extensive

study as it has in the coronary arteries but the evidence available suggests that similar processes are at work in the two sites. Athero-sclerotic occlusion of the lower limb arteries is well known as a cause of " senile " gangrene but less well known is the extent and severity of exactly similar changes in elderly people who are free from symptoms of ischæmia. Rodda (1950) in a detailed study of the lower limb arteries of fifty people with no history of ischæmia found there was a total or nearly total occlusion of one or more arteries in more than 40 per cent. of the elderly ; one artery was occluded in 25 per cent., two in 12 per cent., and three in 4 per cent. Lindbøm (1950), in a similar investigation, found that major arterial occlusion in the lower limb was twice as common as coronary thrombosis. Both authors note the frequent occurrence of medial calcification (Mönckeberg sclerosis) and agree that it has no effect on the arterial lumen. Rodda states that the finding of arterial calcification in skiagrams of the lower limb is valueless as an indication of ischæmic disease because in 90 per cent. of cases it is in the media and does not affect the lumen. Both authors note a lack of correlation between the most frequent sites of atheroma and of occlusion. Lindbøm believes that this is because occlusion is usually due to thrombosis and he regards intimal calcification as evidence of previous thrombosis. It is worth reiterating that these findings refer to people without evidence of lower limb ischæmia. In those who have ischæmic symptoms such as pain on exercise (wrongly called " intermittent claudication ") or gangrene, the essential lesions are similar but of greater severity. In such cases there is often total occlusion of the lower femoral or popliteal artery by thrombosis in addition to the lesions described above. From this short account it is apparent that of the various forms of arterio-sclerosis, the only one which produces disability or death is athero-sclerosis and that it does so largely if not exclusively by associated thrombosis.

The Ætiology of Athero-sclerosis

The ætiology of athero-sclerosis has been studied mainly by animal experiments. Very many procedures have been tried but the only one which has produced lesions resembling human disease is the administration of cholesterol. This undoubtedly produces lesions which histologically are strikingly like human athero-sclerosis but the conditions under which they are produced are so unlike anything occurring in man that the application of such experiments to human pathology has been seriously criticized.

The main lines of criticism are that cholesterol sclerosis can only be produced in rabbits or similar herbivora, that it can only be produced by causing an intense and prolonged hypercholesterolæmia during which the reticulo-endothelial system becomes laden with cholesterol in a manner never seen in humans, and that the lesions have a different distribution from that of human disease. However valid these criticisms, they do not entirely exclude cholesterol as a possible agent in human disease. Cholesterol sclerosis has been produced in omnivora. Schmidtmann (1932), by combining cholesterol feeding with vitamin D overdosing and enforced exercise, produced atheromatous lesions in the coronary arteries of rats. Similarly, Steiner and Kendall (1946), by combining cholesterol with thiouracil produced aortic and coronary atheroma in dogs, and the lesions had the same anatomical distribution as in human atheroma. These dogs certainly had a severe lipæmia and fatty change in their livers but in this respect it is necessary to remember that experimental athero-sclerosis is produced in a few months, whereas its human counterpart takes a large part of a life-time to develop. Anitschkow (1933) claims that by feeding smaller quantities of cholesterol over longer periods it is possible to produce vascular lesions with only minimal fatty change elsewhere.

If cholesterol plays any part in human athero-sclerosis then conditions associated with lipæmia ought to be associated with excessive athero-sclerosis. The evidence in this direction is equivocal ; in biliary obstruction and nephrotic nephritis, both of which are associated with hypercholesterolæmia, athero-sclerosis is neither severe nor widespread ; on the other hand, it is unduly frequent in diabetes mellitus and during the pre-insulin era, when for a period, many patients were treated by a high-fat diet, the incidence of athero-sclerosis in the younger age groups of diabetics rose steeply and was not infrequently complicated by coronary thrombosis. Rabinowitch (1935) has contrasted the incidence of athero-sclerosis in diabetics treated in pre-insulin days by a high fat diet with those treated by insulin and a low fat diet ; he concludes that the lipæmia of diabetes played an important part in the production of athero-sclerosis. Aschoff believed that the accumulation of lipoid material in the arterial walls in athero-sclerosis was due to " imbibition " by the intima of cholesterol ester contained in the arterial blood. Schoenheimer (1946) has investigated this problem by using isotope-labelled fats and has shown that endogenous (metabolic) and exogenous (dietary) fats

are not separate fractions. His results suggest that all fatty substances in the body are in a state of constant flux, their molecules being continually broken down and rebuilt, and that there is no metabolic discrimination between dietary fat and body fat, both being metabolized together irrespective of their source. Dietary fats within a short time after absorption are broken down and their constituent radicles mixed with those of the body fats in a common metabolic "pool." From this pool cholesterol and other lipoids are readily synthesized. It follows from this work that cholesterol as such need not be absorbed provided that there is a sufficiency of molecular building materials. Whether this has any bearing on athero-sclerosis will depend on the method by which fatty substances reach the vessels and some recent observations suggest that the route is a remarkably direct one. In normal people after a fatty meal the blood contains floating fatty globules large enough to be visible by dark ground illumination. These globules are called chylomicrons and can be counted and expressed as numbers per unit volume of blood. Moreton (1948) showed that blood taken during fasting or after a non-fatty meal contained very few chylomicrons, whilst after a fatty meal there was a shower of chylomicrons reaching very large numbers and having a peak three to five hours after the meal. Becker and his colleagues (1949) took this observation a stage further by studying the effects of a standard fatty meal on thirty young and thirty old people. In the former, with a mean age of eighteen years, the fasting chylomicron count was 50 ; three hours after the meal it reached a maximum of 450 and fell to normal in five hours. In the elderly group (mean age seventy-six years) though the fasting count was the same, after the meal it reached a peak of 1,700 at eight hours and did not return to normal for twenty-four hours. In other words, elderly people deal with fats slowly and after a fatty meal the chylomicron count is abnormally high and prolonged. These findings do no more than suggest the route whereby fat enters the vessels. There is, however, evidence from animal experiments that chylomicrons can enter the vessel from the lumen and be deposited in the intima. Hueper (1941) has shown in pigs that a variety of relatively inert foreign substances—polyvinyl alcohol, methyl cellulose, pectin—injected into the blood-stream are deposited in the intima of arteries forming lesions like those produced by cholesterol, and that they apparently enter directly through the endothelium without being carried in by phagocytes. Moreton (1948) investigated these substances and found that

they float in the blood as macro-molecular aggregates similar in size and appearance to the chylomicrons composed of fat. From these observations Moreton postulates that in man circulating fatty globules penetrate the arterial endothelium directly from the blood-stream but are unable to penetrate through the elastic lamina and, therefore, remain in the intima. Here they are phagocytosed and most of the fat broken down and absorbed but cholesterol being more stable remains *in situ*. Since cholesterol forms only a small fraction of the penetrating fat the total amount retained will be small and a considerable time will be necessary to allow any large quantity to accumulate. This hypothesis may possibly account for the milder degrees of intimal fatty change but it is difficult to reconcile it with large masses of lipoid and neutral fat deep in the intima and covered by a thick layer of dense collagen. It is also difficult to correlate such an intra-arterial accumulation of alimentary cholesterol with Schoenheimer's finding that body lipoids are constantly being broken down and re-synthesized.

Whilst the problem of the genesis of athero-sclerosis still remains unsolved, there is little doubt that associated thrombosis is a major factor in the causation of vascular occlusion with consequent ischæmic changes in the organs. Two mechanisms are involved in this thrombotic process. Winternitz and various other workers have shown that hæmorrhage from the vasa vasorum into the thickened intima of an athero-sclerotic vessel is frequently followed by thrombosis in the adjacent lumen. In this case athero-sclerosis is the underlying cause but some other factor may well be responsible for precipitating the hæmorrhage. Lindbøm, from a study of lower limb arteries, has found evidence that the precipitating factor is trauma. He found such hæmorrhages to be remarkably common at sites where vessels are exposed to stretching or acute bending stresses. On the other hand, the investigations of Duguid on the coronary arteries and aorta, and those of Heard (1949) on the renal arteries, show that intramural hæmorrhage is not an essential prelude to thrombosis. They believe that mural thrombi can occur in a vessel without visible underlying damage. Here the mechanism of thrombosis is unknown but a possible explanation is suggested by the investigation of fibrinolysin by MacFarlane and his colleagues (1946) and Mole (1948). Mole suggests that under normal circumstances fibrinolysin is formed by vascular endothelium and dissolves any fibrin clots that may be found, but that in infection and cachexia

fibrinolysin formation is inhibited. This may possibly prove to be an important factor in the genesis of vascular thrombosis.

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CHAPTER IX

DISEASES OF THE LUNG

Introduction

In selecting material for this chapter, the policy has been to include not only major conditions, such as malignant disease, pneumoconiosis and œdema, but also certain pathological processes of lesser importance in which increasing interest has been shown because of the radiological changes which are found to accompany them. Cardiovascular disease may be associated with deposits of hæmosiderin and of calcium in the lungs, and cause shadows which may be confused with those due to other conditions, notably tuberculosis and pneumoconiosis. Correlation of radiological and pathological changes in the lung has shown that disease processes of diverse type may cause shadows which appear very similar in radiographs. Sometimes only detailed study of the distribution of the lesions may allow distinction of such conditions. The chapter ends with an account of a new technique, whereby sections representative of the whole lung are mounted on paper. These are used for comparing pathological with the radiological changes, and they also serve as permanent records which may be stored in book form or filed with the clinical notes.

Primary Cancer of the Lung

Ætiology. Recent information strengthens the likelihood that the cause of lung cancer is exogenous. For a long time the only definite association of this type of neoplasm with environment was in the metalliferous mines of Schneeberg and Joachimstal, where some ingredient in the ores caused a very high incidence of cancer of the lung. The ores in Schneeberg contained radioactive materials as well as arsenic, cobalt, manganese, nickel and other metals. The ores in Joachimstal contained radium, arsenic, cobalt, nickel and silver. Other industries are, however, now known to have lung cancer hazard. Machle and Gregorius (1948) have found that there is a high death rate from cancer of the respiratory system among workers in the chromate industry, the crude death rate from cancer of the lung being twenty-five times that of the normal. There is evidence

that only the monochromates may be carcinogenic, as one plant handling only bichromates and chromic acids did not show cancer of the respiratory system. Cancer of the nasal mucosa and sinuses has been known to occur in high incidence in nickel workers, and this industrial hazard has long been scheduled. In 1949 the schedule was extended to include cancer of the lung (National Insurance (Industrial Injuries) (Prescribed Diseases) Amendment (No. 3) Regulations, 1949). The carcinogenic agent may be the gas, nickel carbonyl.

These few instances of industrial liability to lung cancer are undoubtedly due to inhaled substances, all of them related to metals or their compounds. At first sight they would appear to have little bearing upon the causation of cancer of the lung in the general population, but there is possibly a relationship in the causes. Doll and Hill (1950), in an investigation into the association of smoking with cancer of the lung, found that cigarette smoking was closely related to the incidence of the disease, but they could not deduce a simple relationship between the increased consumption of tobacco and the increased number of deaths attributed to cancer of the lung, and suggest that one possible explanation is that the carcinogen in tobacco smoke is introduced into the tobacco during its cultivation or preparation. They point out, however, that they have no evidence of the nature of the carcinogen, but that Daff and Kennaway have reported that the only carcinogenic substance they could find in tobacco smoke is arsenic. If the latter is the carcinogen, the source of it may be arsenic-containing insecticides used since the end of the nineteenth century. Referring again to the Schneeberg and Joachimstal mines, it may be pointed out that, although radioactive substances have been regarded as the carcinogenic agent, this has not been proved and, alternatively, it has long been suggested that arsenic in the ores may be the cause. Hill and Faning (1948) investigated the incidence of cancer in a factory dealing with inorganic arsenic compounds. They found an increase of cancer of the lung and skin confined to workers in the chemical processes, including engineers and packers, but absent from those unlikely to be exposed to the hazard, such as printers and boxmakers.

Silicosis is unlikely to be a predisposing cause of cancer of the lung. Some authors have described these two conditions together, but these have been small series and the association of the two diseases may have been coincidental. If silicosis were a cause,

cancer of the lung would have appeared among the many thousands of cases of silicosis in the gold mines of South Africa. The evidence is, however, to the contrary. Post-mortem studies by the Miners' Medical Bureau of South Africa (1946) have shown that lung cancer is no more frequent in silicotic miners than in either non-silicotic miners or a comparable number of males in the general population. The respective figures were 0·70 per cent. among 1,438 silicotics, 0·71 per cent. among 1,679 non-silicotic miners, and 0·93 per cent. in 1,393 males who had not been miners. Likewise, Vorwald and Karr (1938) and Gardner (1940) from the radiological examination of large numbers of cases of silicosis found no evidence of predisposition to carcinoma. Asbestosis, on the other hand, is frequently followed by cancer. Kennaway and Kennaway (1947), from the analysis of death certificates for the years 1921–38 showed that no occupations involving exposure to any kind of dust except asbestos, arsenic and nickel caused increased incidence. The coal mining industry and other occupations in which there is a liability to silicosis do not show an increased incidence. Workers exposed to coal-gas and tar show an increased incidence, which in the latter part of the period studied, however, did not exceed two and a half times that of the general population. No special occupations were found to which could be attributed the increase in the total number of cases certified as dying from cancer of the lung in the general population. Certain open-air occupations where there is exposure to the dust of roads were associated with an increase, but there was no evidence that the general increase was caused by tarring of the roads. Stocks (1947) has shown that there is a higher mortality from cancer of the lung in towns, and this is compatible with such an ætiological factor as smoke. Further investigation may be expected to show whether the general air pollution is less potent than cigarette smoke in producing pulmonary cancer.

Diagnosis. Early diagnosis of cancer of the lung is of obvious importance as surgery, to be successful, must be carried out before the disease is advanced. Laboratory diagnosis includes histological examination of biopsies and cytological examination of sputum or bronchial secretion. Biopsies are usually obtained through a bronchoscope and less commonly by aspiration through a needle introduced *via* the chest wall. Some experienced histologists are apparently not convinced of the usefulness and accuracy of cytological examination of sputum, so that further reference

to this topic is justified. Among the more important articles on the subject are those contributed by Dudgeon and Wrigley (1935), Gloyne (1937), Barrett (1938), Wandall (1944) and Farber and his colleagues (1950). The latter publication includes a detailed account of 1,526 cases, and they suggest that when the growth is large enough for biopsy it is probably beyond the scope of surgical cure ; they therefore emphasize the importance of the examination of sputum. The accuracy of diagnosis varies with the experience of the pathologist, but when learnt the methods are of great value to the surgeon. Examination may be made either by the wet film method or by centrifuging the deposit, fixing, embedding and cutting sections. The wet film method is usually preferred. The best techniques are those of Dudgeon and of Papanicolaou—the latter being the more popular in the U.S.A.

Our own experience is that cytological examination is useful, and can be reasonably accurate in the hands of an experienced person. It is generally agreed that it should not replace biopsy, but should be used especially in those cases where the suspected growth is beyond the reach of the bronchoscope, or when the material obtained by bronchoscopy is not satisfactory or gives a negative result. The sputum examination has the advantage that it can be repeated without distress to the patient. The ability to recognize malignant cells can only be acquired by extensive study of sputum from patients with cancer and also from those with a wide variety of diseases which are not malignant Critics of the technique have remarked that illustrations accompanying reports do not show sufficiently distinctive characteristics between malignant and non-malignant cells ; such differences are often difficult to show in photographs, although the coloured photomicrographs of Farber and his co-workers are a useful guide. Accuracy in diagnosis can only be acquired by familiarity with the preparations themselves. Details of the various techniques will not be repeated here, but important points may be emphasized.

The sputum should be fresh, the smears made as soon as possible and fixed within a few seconds of spreading as even the slightest drying may blur the cytological details. Farber, using the Papanicolaou technique, states that if the specimen cannot be sent immediately to the laboratory it should be smeared and the films fixed for about two hours, after which they may be allowed to dry. They may then be kept for several days without ill effect. Schuster (1947) mixes sputum with a solution of methylene blue and glycerine on a slide. The method is quick but the preparations are not permanent.

The appearance of individual malignant cells in sputum often differs from the appearance in fixed tissue sections, but when occurring in clumps or in small tissue fragments similarity may be close (Figs. 9/1 and 9/2). The accompanying drawings (Figs. 9/1–9/6) are from preparations made by Dr. F. R. Magarey from patients treated at Sully Hospital. The Dudgeon technique was used. When examined under low power, malignant cells can usually be picked out by their hyperchromatism, and the preparations should be screened for such cells. When examined under high power additional important features are the relatively large size of the nuclei to the cytoplasm, the irregularity of nuclear outline and the frequency of multiple nucleoli (Figs. 9/2, 9/3). When the cells are in groups, variation in size may be marked and " cannibalism " of one cell by another may be present, giving the so-called " bird's-eye " appearance (Fig. 9/4). In anaplastic carcinomas most of the cells show little or no cytoplasm, the nuclei vary in shape and size and may have large prominent nucleoli (Fig. 9/5). Anaplastic cells are recognized more often in secretions aspirated from bronchi than in sputum. In squamous carcinoma the nuclei are larger and more irregularly shaped than in the cells of normal smears. The recognition of adenocarcinoma as a separate type is difficult. Vacuolation of the cells, which has been interpreted by some authors as proof of adenocarcinoma, was seen in some cases which were subsequently shown to be squamous carcinoma (Fig. 9/6). By cutting large sections of their cases of adenocarcinoma, Phillips, Basinger and Adams (1950) found all of them to be partly squamous, although not keratinized. Sometimes vacuolated cells were found in the same smear as typical squamous carcinoma cells.

Examination of material aspirated directly through the chest wall in 56 cases of carcinoma gave a positive diagnosis of cancer in 44 (Gledhill *et al.*, 1949) ; these findings were subsequently confirmed at autopsy or by other means. The tumour was located by radiograph, the aspirated tissue was embedded in paraffin and stained with hæmatoxylin and eosin. Smears were also examined. The aspiration procedure was accompanied by a mild degree of pneumothorax in three instances and slight hæmoptysis in five. The method is nevertheless claimed to be safe and a valuable aid in diagnosis, although admittedly serious complications may occasionally occur. Rosemond, Burnett and Hall (1949) advise aspiration biopsy only when bronchoscopy and sputum examinations are negative. They recommend it particularly in circum-

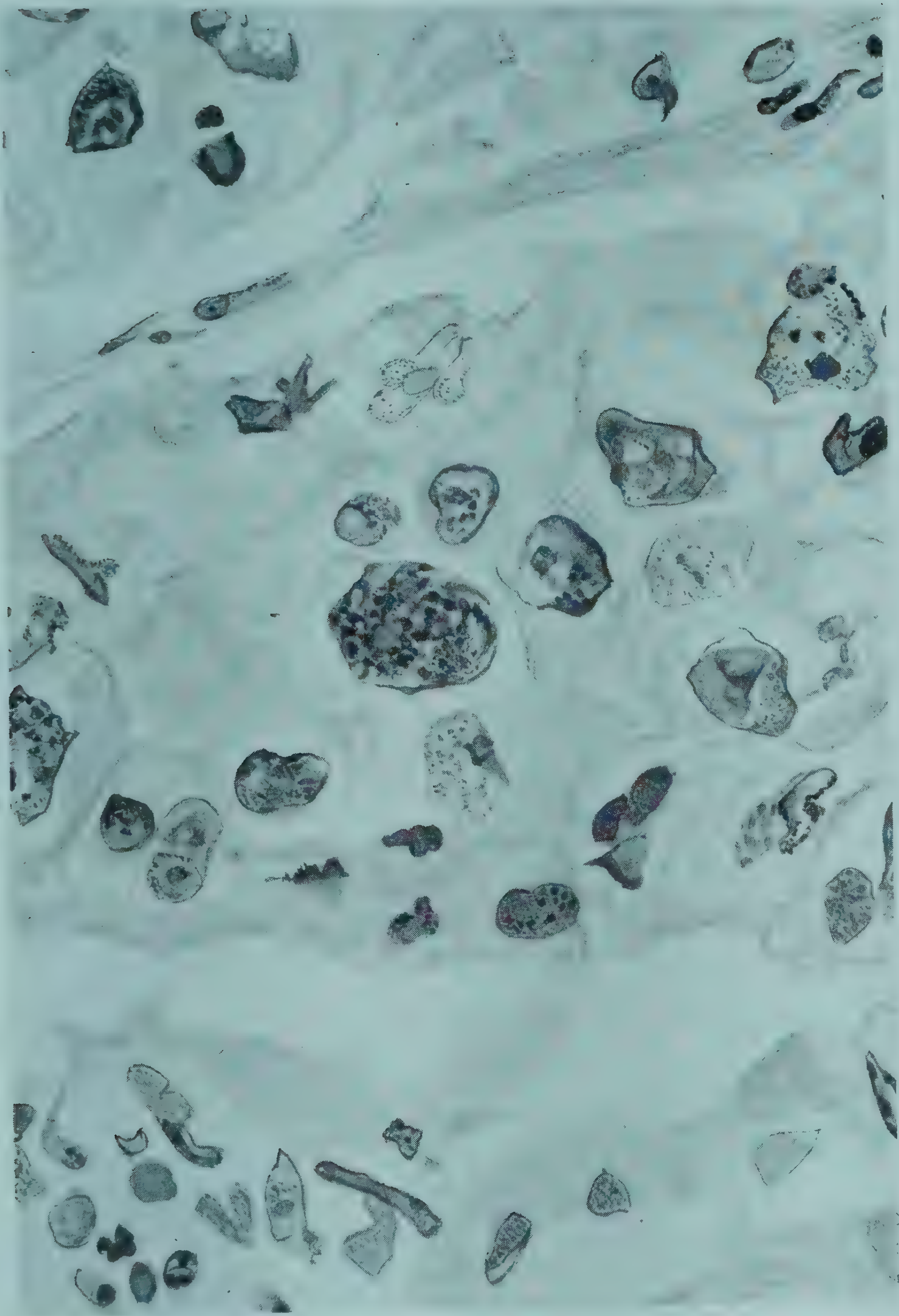

FIG. 9/1. Paraffin section of lung tumour. × 800. Sputum from this case is shown in Fig. 9/2.

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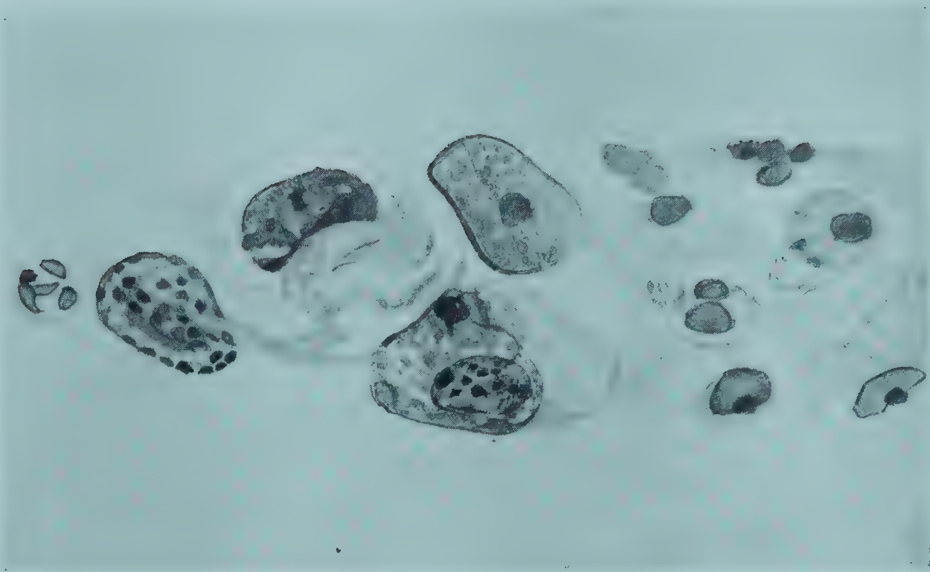

FIG. 9/2. Sputum from case of squamous carcinoma of lung. × 1200.

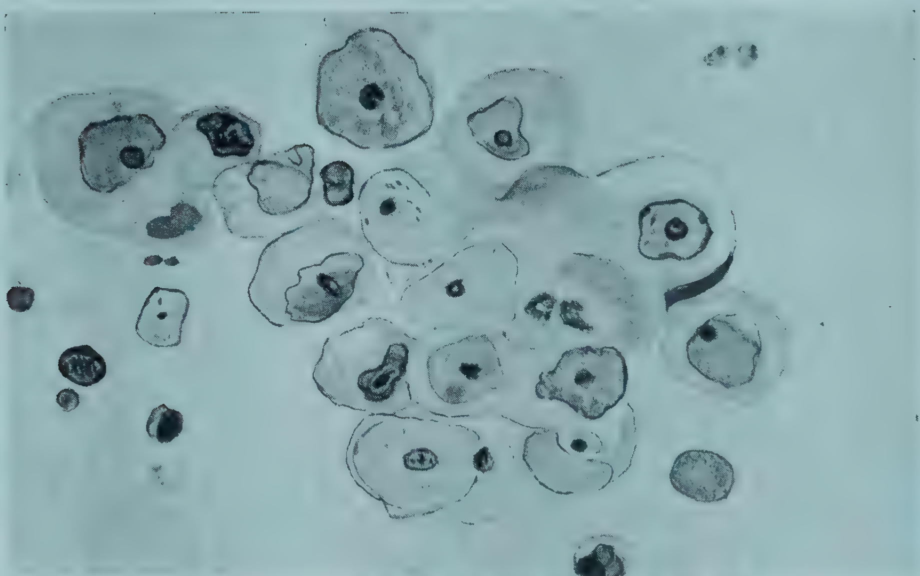

FIG. 9/3. Sputum, squamous carcinoma of lung. × 900.

Fig. 9/4. Sputum, squamous carcinoma of lung showing inclusion of one cell within another. × 1500.

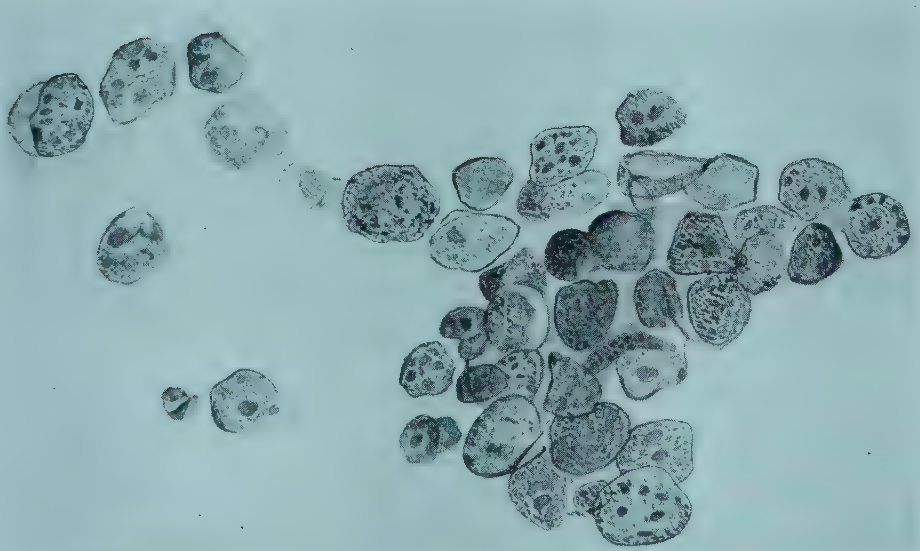

Fig. 9/5. Sputum from case of anaplastic carcinoma of lung showing cells with little cytoplasm ; variation in shape and size of nuclei and large prominent nucleoli. × 1000.

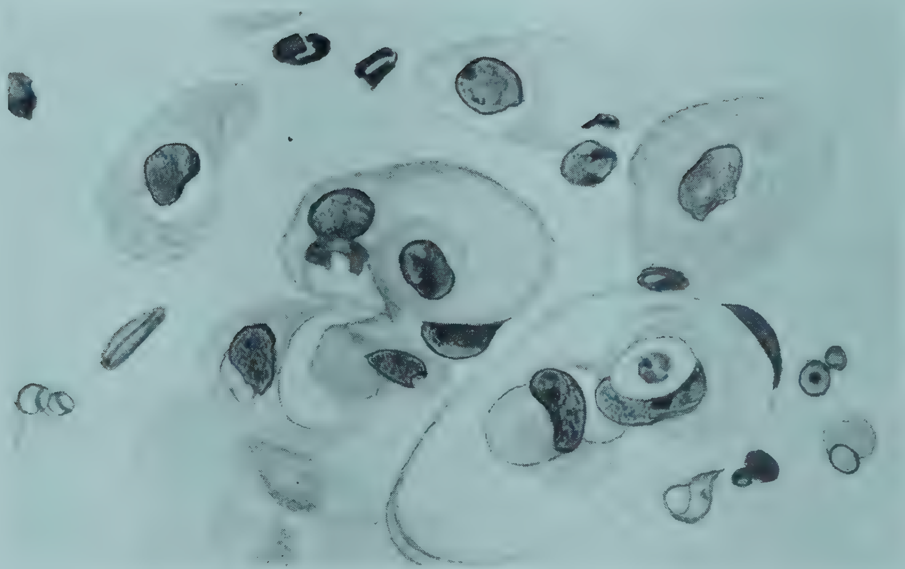

Fig. 9/6. Sputum from case of squamous carcinoma of lung. There is vacuolation of cells and "cannibalism" is pronounced. × 1200.

scribed peripheral lesions. The needle is introduced into the tumour under radiographic examination. They used this method in 220 cases of suspected carcinoma and 135 were proved to be positive. In no instance did the tumour spread along the needle track, but pneumothorax sometimes occurred and caused the death of one patient. The method is evidently not without danger, although these authors state that the risks are comparatively small.

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Pulmonary Œdema

Pulmonary œdema may arise from a variety of causes, but it appears ultimately to be due to increased permeability of the pulmonary capillaries allowing protein-rich fluid to escape through their walls (Cameron, 1948). The mechanisms leading to such capillary disturbances are, however, very varied and often complex. Recent contributions to the elucidation of these mechanisms have been made by Drinker (1945) and by Cameron.

The latter has also reviewed the past work and indicated what has been established and what remains uncertain. Leaving aside œdema due to infection, he described the condition under three main headings : circulatory failure ; nervous disturbances ; and inhalation of irritant gases.

Œdema of Circulatory Failure. The most important of the cardio-vascular diseases causing pulmonary œdema are systemic hypertension, mitral stenosis and coronary occlusion, and the most popular explanation of the œdema is dissociation of output from the left and right ventricles. Cameron accepts the emphasis placed upon the part played by failure of the left ventricle, but is not convinced that this mechanical disturbance alone accounts for the pulmonary œdema. He criticizes much of the experimental work which has been done in the past and stresses that careful animal experiments have shown that marked changes in the systemic blood-pressure have but little immediate effect upon the pulmonary arterial pressure (Johnson *et al.*, 1937). He points out that the results of experiments producing obstruction of the pulmonary flow certainly lead to the conclusion that œdema of the lungs can be produced by such obstruction, but only if the disturbance in circulation brings the animal near to death. Another investigation (Cameron and De, 1949) was made to determine whether lung œdema could be brought about in animals through interference with blood-flow by severe pulmonary embolism. This was produced by injecting starch granules into the veins, and, although a marked fall occurred in the carotid pressure, together with a rise in the right auricular pressure and almost complete interruption of the pulmonary blood-flow, there was no evidence of œdema.

Cameron looked for factors which might act in association with pulmonary hypertension in producing œdema, and like other observers he has been impressed with the frequent history of dyspnœa which precedes the crisis of pulmonary œdema, and he regards respiratory embarrassment as playing an important part in the cause of the œdema. Drinker (1945) agrees that anoxia is an important contributory factor in causing pulmonary œdema. In experimental animals he likewise found that whereas increased pressure in the pulmonary capillaries does not readily cause œdema, superadded anoxia will precipitate it. As œdema itself leads to further anoxia, the prevention of progressive pulmonary œdema clearly rests upon early and adequate administration of oxygen. He stresses also the action of the movement of the

lungs in prevention of œdema and advocates carbon-dioxide and oxygen during anæsthesia to prevent anoxia and also to stimulate respiratory movements.

So-called "Uræmic Œdema." In patients suffering from hypertensive heart failure with uræmia, Roubier and Plauchu (1934) reported a characteristic radiographic appearance which they ascribed to "subacute œdema." Several writers have confirmed these findings. In those patients who showed clinical improvement, the shadows disappeared within a few days, attributed by some observers to a fall in blood urea and by others to relief of the heart failure.

Doniach (1947), finding a paucity of pathological description in previous reports, investigated in detail the autopsy findings in 5 such cases. Radiographs taken during life showed fine or gross mottling which had a typical distribution involving the mid-lung zones and radiating from the hilar regions with clear areas at the apices and bases. He found the distribution was characteristic. It was unlike that seen in the usual œdema of chronic heart failure, which produces loss of translucency particularly in the basal zones. The severity of the radiological changes was out of proportion to the minimal physical signs in the chest, which may be explained by the central distribution of the œdema. Clinically the condition was not infective in nature, and none of the five patients in Doniach's series was febrile. Most of the patients showed left ventricular failure in the form of paroxysmal nocturnal dyspnœa, normal or moderately raised jugular venous pressure and very little if any peripheral œdema. All showed high blood urea, 238 mgm. per 100 ml. or more, but there was no obvious correlation between the onset of œdema and the occurrence of biochemical disturbances associated with uræmia such as acidosis or hypoproteinæmia. At autopsy there was widespread solid œdema in the lungs. Microscopically the exudate was found to contain a greater or lesser amount of strongly eosinophilic fibrinous material in the terminal air spaces. This was associated with mononuclear infiltration and organization. Albuminous rather than fibrinous œdema is more frequently recorded in uræmia, and Doniach concluded that more instances must be studied before it is possible to determine whether there is a fibrinous element in all and what are the relative frequencies of the predominantly fibrinous or albuminous types. In no case was there any evidence in the lungs of arteritis or of pneumonia. He concluded that the cause is cardiac failure and suggests that in

uræmia congested alveolar capillaries may be so altered as to leak coagulable fluid with only a moderate rise in pulmonary capillary pressure. Anoxia is a marked feature in these cases, and this has been attributed to the exudate interfering with the gaseous exchange.

Pulmonary Œdema of Nervous Origin. Cameron and De (1949) have thrown a good deal of light on the association of intracranial disturbances and pulmonary œdema by experiments in which a fibrin-forming mixture was injected into the basal cistern of rats and rabbits. After one to five minutes a profuse, frothy fluid rushed from the nose and mouth and the animal died. Asphyxia apparently plays no part in the development of this particular form of pulmonary œdema. The lungs showed severe acute œdema, which microscopically was seen to be composed of an eosinophile exudate rich in protein and containing red blood corpuscles. If the vagi were divided or paralysed by atropine the phenomenon did not occur. The injection of fibrin into the cistern was associated with a rise in the carotid arterial pressure, falling gradually to zero at death, together with a rise of the pressure in the right auricle. Records of the arterial and of the right auricular pressures showed no differences in the animals which developed the œdema and in those which did not. The changes in vascular pressure were evidently not the cause of the œdema as the latter did not occur when the vagi were cut, although the arterial and auricular pressures rose as before. The stimulus for the œdema arose within the central nervous system as the substances were not active when injected into other parts of the body. The stimuli from the brain modified the permeability of the lung capillaries so as to allow plasma to leave them and produce œdema of the lung. It could be shown that a general rise of intracranial pressure was not the cause of the neurogenic œdema, but the site of a local stimulus was uncertain. Cameron and De found that the fibrin injected into the cistern entered the fourth ventricle and extended along the aqueduct of Sylvius and into the third and lateral ventricles, and a thin film sometimes tracked across the cerebral hemispheres. Since the fibrin had this wide distribution it could not be decided how or where the brain was being stimulated, although among the possibilities was that of contact of the fibrin with the vagal nuclei.

Chemical Causes of Pulmonary Œdema : Inhaled Gases. Œdema due to irritant gases such as phosgene and ketene has been studied by Cameron and Neuberger (1937), who found that these

substances have a direct action on the protoplasm of the lung capillaries, making them extremely permeable to blood plasma, which fills the air spaces and drowns experimental animals. It has been difficult to detect structural changes in the vessel walls in phosgene poisoning, but Short (1942) has shown that the mitochondria of the endothelium break up and disappear before the capillaries leak.

Substances Acting after Absorption. Pulmonary œdema may also be produced by substances which act after absorption into the circulation. Many such substances have been known in the past, but compounds related to thiouracil have been found particularly effective. Jones (1946) found that four compounds, α and β naphthyl thiourea and α and β dinaphthyl thiourea, produce massive pleural effusions and pulmonary œdema which sometimes cause death. The only pathological changes were in the respiratory system and in the thyroid gland. There was no impairment of the circulatory or nervous function during life, and the condition appeared to be due to an action on the cells of the thyroid gland. These chemical substances appear to act on the permeability of the lung capillaries without affecting those elsewhere.

The Rôle of Infection. It is well recognized that œdematous lungs have a predisposition to infection and experimental work (Harford and Hara, 1950) suggests that the explanation may be that the œdema fluid provides a suitable culture medium for bacteria. It was found that in mice, infection with influenza virus induced susceptibility to secondary pneumococcal pneumonia. These workers noted that the virus infection was associated with pulmonary œdema and they carried out experiments to imitate this condition, without producing a primary infection. Sterile mouse serum was introduced into the bronchi and the pneumococci were inhaled in fine droplets. Of 19 mice treated in this way, 17 died of pneumonia ; in 18 controls in which a bronchial cannula was passed, or saline injected before inhaling the pneumococci, only 1 died. The authors considered that the œdema fluid does not act merely mechanically by facilitating transport of infection through the lung by movement of the fluid, but provides nutrient medium, and in addition delays the appearance of phagocytes. The thesis that œdema fluid is important in providing a medium favourable for bacterial growth is more satisfying than any hypothetical reference to lowering of the individual's resistance. These experiments may have little

bearing upon human infection, but they are suggestive when it is appreciated, as Cameron has pointed out, that in œdema of the lungs from many causes the fluid exuded is rich in protein. Moreover, these fluids are not readily removed from the lung by normal lymphatic drainage. Courtice and Phipps (1946) have shown that saline introduced into the lung is quickly absorbed directly into the blood-stream, but serum is absorbed far more slowly by the lymphatics.

To conclude, it may be repeated that the presence of much protein in pulmonary œdema fluid does not necessarily indicate an inflammatory cause, as such fluids may occur in the œdema of mechanical origin. The protein when it coagulates may form membranes which line the respiratory bronchioles and atria, obstruct the openings of their tributary alveoli and interfere with respiratory exchange. The presence of much protein in the transuded fluid may perhaps explain the tendency for infection to develop in œdematous lungs.

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Pulmonary Changes in Congestive Heart Failure

Pulmonary Hæmosiderosis. The presence of hæmosiderin in the lungs in chronic heart failure has long been recognized, but only recently has it been appreciated that this pigment may accumulate in amounts sufficient to produce radiological changes. The most striking examples are in association with mitral stenosis. The opacities are of such shape and density as to simulate the appearances of pneumoconiosis, miliary tuberculosis and sarcoidosis (Fig. 9/7). The hæmosiderin accumulation is multifocal, the individual foci being up to about 3 mm. in diameter (Fig. 9/8).

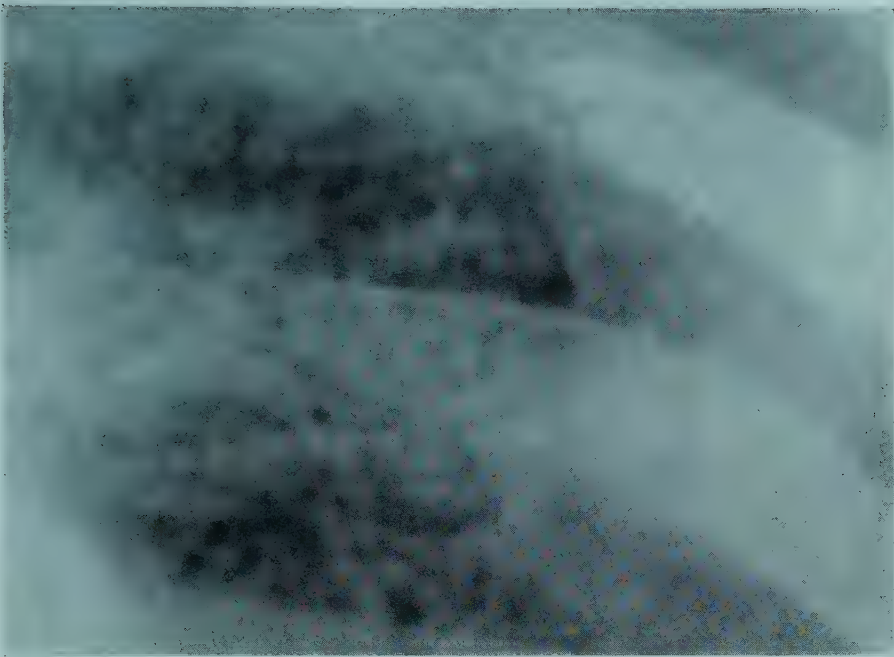

FIG. 9/7. Radiograph of chest during life of a woman with mitral stenosis. $\times 1\frac{1}{2}$.

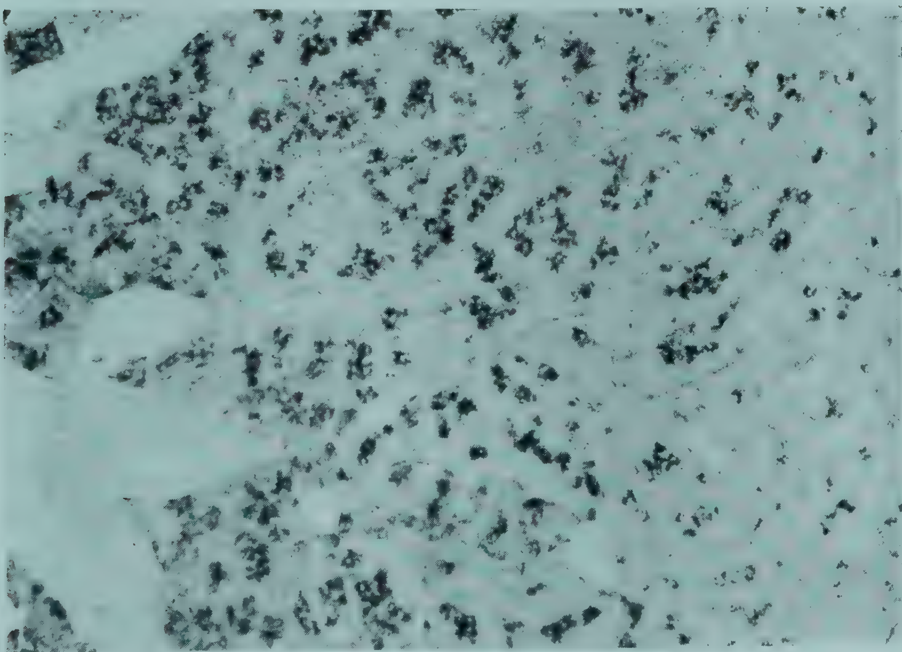

FIG. 9/8. Section of lung corresponding with area in Fig. 9/7 showing deposits of hæmosiderin. $\times 1\frac{1}{2}$.

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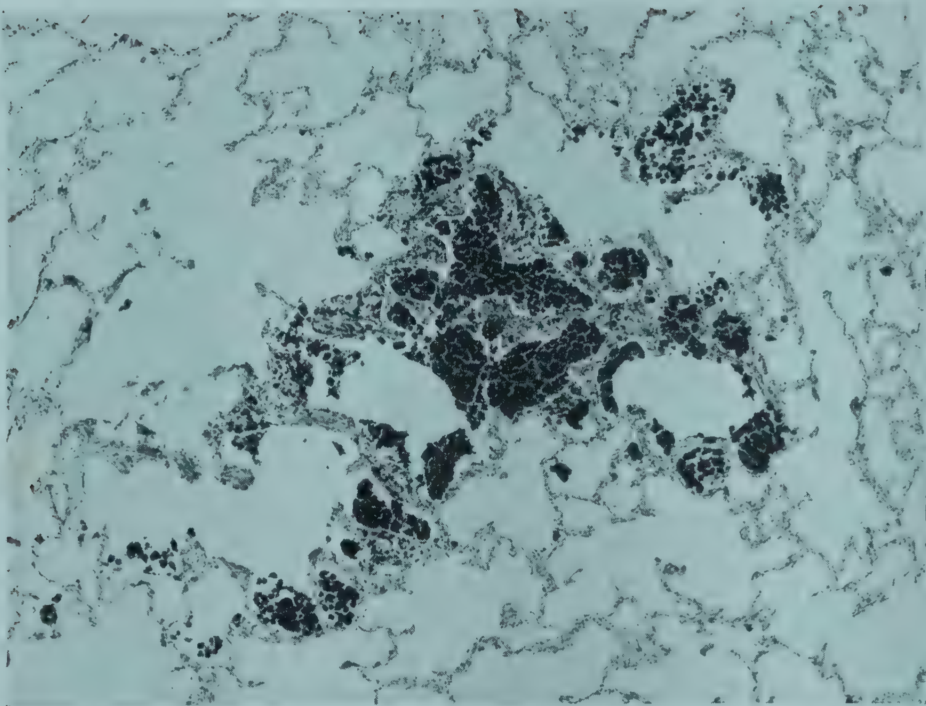

FIG. 9/9. Lung from a case of mitral stenosis. Alveoli packed with hæmosiderin-containing phagocytes. × 600.

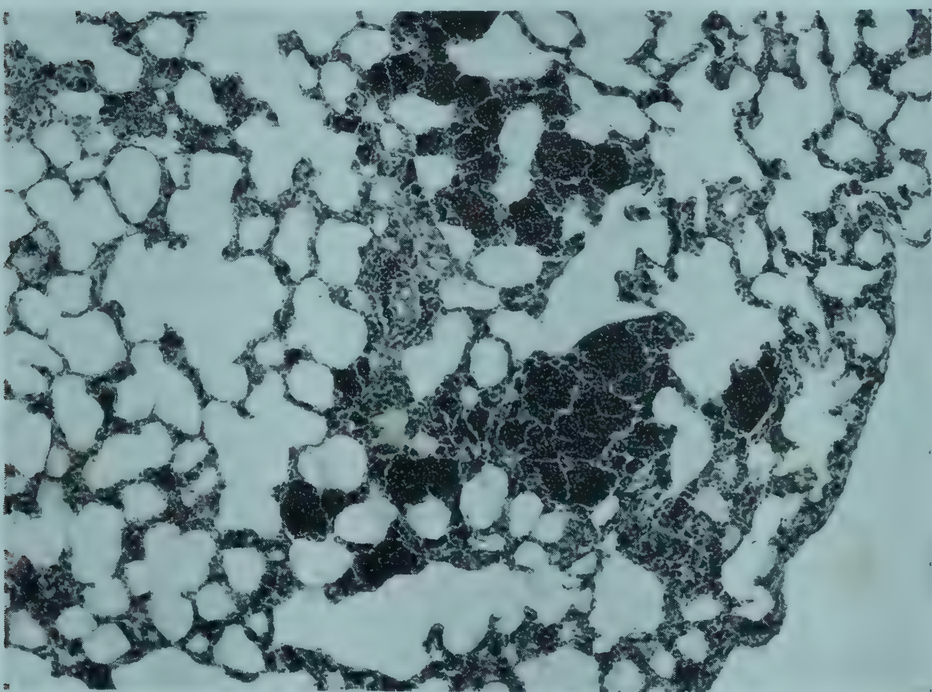

FIG. 9/10. Lung of rat which had received intratracheal injections of blood. The appearances are similar to those seen in mitral stenosis. × 110.

A detailed study of the morbid anatomy of this form of hæmosiderosis (Lendrum, 1950 ; Lendrum *et al.*, 1950) has shown the individual foci as nodules composed of four to twenty adjacent alveoli filled with hæmosiderin which is contained in engorged siderophores (the so-called " heart failure cells "). Each nodule is sharply outlined because surrounding alveoli contain few or no siderophores. The septa within the nodules are thickened and the musculo-elastic tissue of the alveolar ducts hypertrophied. Ferrous impregnation of the elastica of the small arteries occurs, due to the local excess of iron. Around the affected elastic tissue there is giant-cell reaction, even to the extent of the development of giant-cell granuloma. These changes are found to be limited to the region of the hæmosiderin foci, and it is concluded that this is the lung condition described in the past as diffuse brown induration, but it is emphasized that the latter is due to a focal condition and there is no evidence of a generalized fibrosis.

These authors consider that the hæmosiderosis is the result of long-standing right ventricular preponderance. It is seen most prominently in mitral stenosis, but may also occur in patients with systemic hypertension. They explain the focal nature of the hæmosiderosis as due to local hæmorrhages at the specific points of anastomosis between the bronchial and pulmonary arteries in relation to the terminal bronchioles. This explanation presupposes that the sites of hæmorrhage and hæmosiderin accumulation are identical. Such an assumption is at least doubtful, as is at once apparent from a consideration of the fate of inhaled carbon particles, for although these may have a diffuse initial distribution they soon exhibit distinct focal aggregation. An alternative to Lendrum's explanation is that the source of hæmorrhage can occur anywhere in the lung and red blood cells having reached the terminal air spaces are disintegrated and the pigment taken up by phagocytes which migrate in the lung substance to the bifurcations of the respiratory bronchioles, behaving in the same way as phagocytes bearing dust. There is indeed a close similarity in the initial focal distribution of carbon and of hæmosiderin, and there seems little doubt that congregation of phagocytes to certain areas accounts for their focal distribution, although the final quantitative disposition of hæmosiderin and of dust is somewhat different. Carbon is seen principally in the interstitial tissue, whereas hæmosiderin is mainly in the alveolar spaces, as was emphasized by Lendrum. Carbon is carried further along the bronchial tree and, as is well known, much of the dust is

deposited in the hilar lymph-glands, whereas only a little hæmosiderin is found in these glands, even in severe hæmosiderosis of the lungs. This may be due to hæmosiderin going slowly into solution and being absorbed. The fact that there is impregnation of elastic tissue with iron supports such a conclusion, as solution of the hæmosiderin would appear to be a pre-requisite to such impregnation of the elastica. Gumpert (1947) considers that the hæmoglobin disintegrates and the hæmosiderin is taken up by phagocytes which engulf it in the same way as inhaled particles of fine dust. He considers that the phagocytes travel by lymphatics to local aggregations of lymphoid tissue scattered throughout the lung substance, but this movement is not so readily achieved as in pneumoconiosis because of the chronic pulmonary venous congestion. As shown in Fig. 9/9, the bulk of the hæmosiderin is in the alveoli and not in the lymphoid foci.

Further confirmation that transport by phagocytes to definite points accounts for the focal nature of the deposits was obtained by Magarey (1951) in experiments using rats into which blood from the same species was introduced *via* the trachea into the lungs. The blood cells disintegrated and crystalline hæmoglobin appeared in a few days. Thereafter the pigment was taken up by phagocytes which collected into foci at the divisions of the bronchioles, closely imitating the condition seen in the human lung (Fig. 9/10). This blood pigment, at first diffuse in the lung was collected by phagocytes which herded together into alveoli near the bronchioles. Here they remained penned at least for many months. From these experiments it may be concluded that, although Lendrum and his co-workers may be correct in the belief that the source of hæmorrhage in mitral stenosis is from pulmonary and bronchial anastomoses, they are not justified in this conclusion merely because the accumulations of the pigment are focal.

Incidence. Lendrum and his colleagues (1950) examined the lungs from 33 cases of mitral stenosis, 5 of whom had in addition stenosis of the tricuspid valve, and these had no hæmosiderosis of the lungs. Of the remaining 28 cases, 26 showed various degrees of focal deposits of hæmosiderin. Following radiographic examination of a series of patients, it was concluded that a small proportion of those with mitral stenosis will show easily recognizable hæmosiderosis ; a further number will show less definite changes, which are clearly seen when the lungs are X-rayed post-mortem ; and in a third group the hæmosiderosis is of lesser

degree and can only be diagnosed at autopsy by naked-eye or microscopic examination.

In describing 6 cases of hæmosiderosis due to mitral disease, Pendergrass and his fellow-workers (1949) point out that the lung macrophages rarely form clumps large enough to appear as nodular shadows in radiographs. They indicate, however, that in their cases the radiological picture simulated pneumoconiosis, and they compared the changes with those seen in exogenous siderosis due to iron dust, silicosis and miliary tuberculosis. Meiklejohn (1949) reported that a coal-miner with mitral stenosis was shown at autopsy to have pneumoconiosis and hæmosiderosis, but the two types of foci were not distinguished from each other by radiography during life.

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Idiopathic Pulmonary Hæmosiderosis

This condition, first described by Ceelen in 1931, is associated with repeated intra-pulmonary hæmorrhage, resulting in heavy deposits of hæmosiderin in the lungs. A review and summary of the literature of this rare disease (Wyllie *et al.*, 1948) includes records of only 17 cases, all but 1 fatal and all save 1 in children ranging from a few months to sixteen years of age ; the authors added 7 cases of their own seen in the period 1938–47, of these 2 have died. The symptoms are described as consisting of recurrent attacks, often sudden in onset, of fatigue, cyanosis, pallor, increasing dyspnœa and acceleration of the pulse rate. Cough, often followed by vomiting, occurred and at times traces, or even considerable quantities of blood, were present in the sputum or vomit. Each attack lasted from two to several days, or longer, and towards its termination there was increasing pallor and in some instances jaundice ; liver dullness was increased and in 3 cases the spleen became palpable. The radiographs of the chest were often grossly abnormal, commonly having mottled shadows most marked in the hilar areas, and a diffuse speckling

throughout the lungs. The radiographic appearances frequently had a superficial resemblance to those of miliary tuberculosis, but in place of homogeneous foci there were small clear circular spaces surrounded by thickened opaque walls. There was much similarity in this picture and that presented by sarcoidosis and by Gaucher's lipoidosis involving the lung.

On histological examination the air sacs and alveolar walls were heavily infiltrated with hæmosiderin-laden phagocytes and free hæmosiderin. The capillaries in the alveolar walls were thickened and there was an increase of the fibrous elements in the inter-alveolar septa, whilst elastic tissue was far below normal in amount. Fragments of elastic tissue were coated with iron pigment and there was associated foreign body giant-cell reaction. No hæmosiderin was found in other organs.

The cause has not been determined. There is no evidence of a primary hæmatological abnormality and the condition is distinct from hæmochromatosis. Auto cold agglutinins developed, but this change was secondary and interpreted as due to an antibody response to destruction of the red blood corpuscles in the child's lungs. There was no increased fragility of the red cells and increased serum bilirubin and urinary bilinogen was explained by the fact that blood was not shed outside the body, but into the lungs. Most authors have suggested that there is a primary defect in the pulmonary vessels, particularly involving the elastic fibres. Nancekievill (1949), however, found no evidence of deficiency in the elastic tissue of arterioles or alveoli, and he considers that the changes in the elastic fibres are the result rather than the cause of the hæmosiderosis.

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Ossification of the Lung in Cardiac Disease

Areas of circumscribed ossification, usually measuring up to about 4 mm. in diameter, occur in the lungs in some cases of mitral stenosis. Elkeles and Glynn (1946) have described an example of this condition and discussed its significance. The radiographic appearances are characteristic, and in their case and in those reported by others, the nodular opacities are in the lower

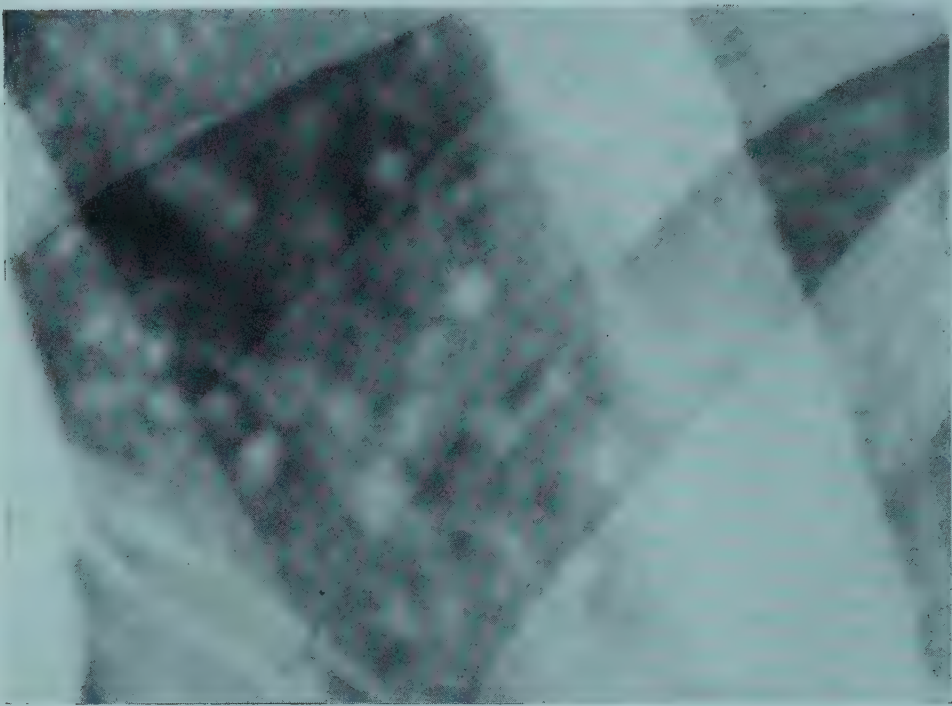

FIG. 9/11. Lower lung field radiograph. Foci of ossification in mitral stenosis.

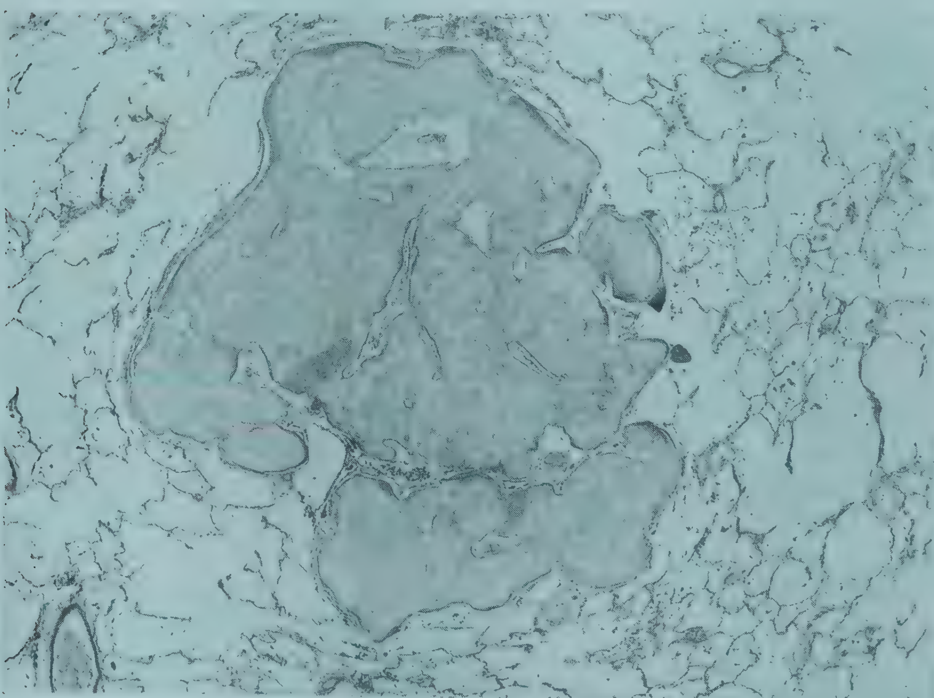

FIG. 9/12. Focus of ossification in mitral stenosis. × 25.

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lung fields and do not involve the apices (Fig. 9/11). Histological examination shows that the nodules consist of bone situated within the alveolar ducts and alveoli. These authors suggest that the condition is a late result of the changes of rheumatic fever, the bone formation taking place in the pulmonary exudate. It should be noted, however, that there is no surrounding fibrosis (Fig. 9/12), and at autopsy the nodules are easily separated from the lung tissue.

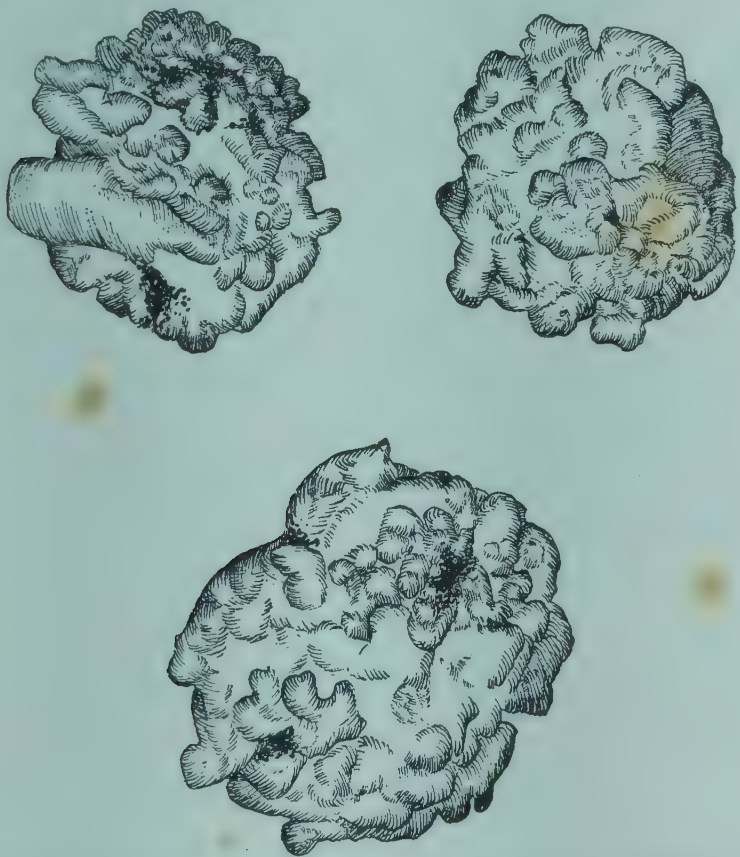

FIG. 9/13. Bone nodules from lung in a case of Mitral Stenosis. ca × 12.

Lendrum and his colleagues (1950) found osseous masses in 3 of their 26 cases of mitral stenosis. They observed that the disposition of the bone resembles that of the fibrinous casts seen in the alveolar ducts and projecting into the alveoli in cases of left ventricular failure and similar to the exudate described by Doniach in so-called " uræmic œdema."

The drawing (Fig. 9/13) shows several bony nodules removed

from the basal zone of a lung of a woman with mitral stenosis. The bone constitutes a cast of the air spaces and shows clearly the alveolar outlines. The dark areas in the drawing are adherent blood. Elkeles and Glynn emphasize that these pulmonary nodules are of practical importance, as they may be mistaken for other conditions, particularly tuberculosis. In mitral stenosis, however, the foci are characteristically basal in distribution. A mistaken diagnosis might prejudice against surgical operation for the mitral disease. The foci are usually not very numerous and unlikely to cause appreciable functional disturbance.

Sahn and Levine (1950) have discussed the differential diagnosis of pulmonary " nodules " seen in radiographs, including those due to histoplasmosis and coccidiodomycosis, which account for a high proportion of cases of pulmonary calcification in some parts of the U.S.A. Two types of radiographic appearance in the lungs in mitral stenosis are mentioned, one consisting of soft areas of infiltration and the other of calcified areas. It is suggested that these two conditions are due to the same basic changes, but this is very improbable. Their observations were based only on clinical and radiological findings, but examinations of lungs from such cases show that, although the two lesions may be present in the same lung, they are quite distinct in their nature, the one being due to hæmosiderin and the other to bone. Elkeles and Glynn state that in their case no iron was present in the osseous tissue.

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Mikrolithiasis Alveolaris Pulmonum

Not infrequently in sections of lung, especially where there has been chronic inflammation, rounded bodies having a laminated appearance may be seen in the air spaces, some staining lightly with hæmatoxylin and others more darkly. Older descriptions refer to them as corpora amylacea ; occasionally they are calcified and resemble the calcispherites seen in other situations. In rare cases these bodies may be present in enormous numbers and occupy most of the alveolar spaces. The first example of the severe form was described by Puhr (1933) under the name " Mikrolithiasis alveolaris pulmonum." He distinguished it from the

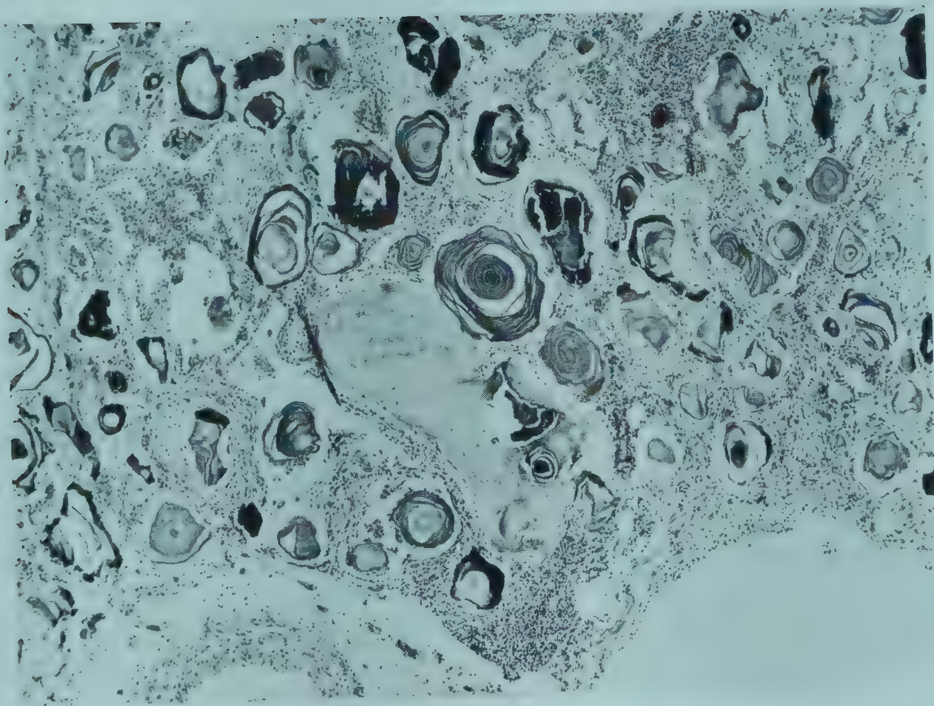

Fig. 9/14. Mikrolithiasis alveolaris pulmonum. × 50.

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more common metastatic calcification on the grounds that in the latter calcium is found in the elastic and collagenous tissue, particularly in the vessel walls, whereas in his case the deposits were intra-alveolar and any thickening and sclerosis of the alveolar walls he considered to be a secondary change. There was no calcification of other organs and no resorption of calcium from the bones ; he interpreted the condition as due to failure of normal excretion of calcium. The actual mode of formation appeared to be due to an encrustation of calcium salts, principally calcium phosphate, on fragments of organic material, probably remnants of inflammatory exudate due to the pneumonia which his patient had suffered on several occasions ; the laminated concretions in the air spaces and also bone formation in the interstitial tissue are shown in Fig. 9/14. Leicher (1949) also described a typical example of the disease and included a summary of the literature.

The condition may be recognized during life because of the remarkable radio-opacity affecting the greater part of both lungs. The apices are the least involved, but the rest of the lungs may be so densely calcified that even when the X-ray exposure is increased many times the outlines of the ribs and the spine are hardly recognizable where they overlie the affected lung. The heart shadow is similarly obscured. Respiratory failure ultimately occurs, but it is surprising how long the patient's respiratory function is maintained. At autopsy the lungs are extremely heavy and can be cut only with great difficulty. The texture is like that of pumice stone and the sawn surface feels like sand-paper. The tiny concretions may be washed out of the lung by macerating with water when they feel and look like grains of sand. In Puhr's case the lungs together weighed 3·5 kg. The dried weight was 58·3 per cent. instead of the normal approximately 20 per cent. ; 74 per cent. of the dried substance could be recovered as ash, mostly calcium phosphate. In Leicher's case the lungs also weighed about 3·5 kg. ; they contained 66·5 per cent. calcium phosphate and 8·6 per cent. calcium carbonate.

This disease appears to be a distinctive form of lung calcification and is unlike metastatic calcification in that it is primarily and mainly intra-alveolar ; the cause is unknown, except that the deposits seem to occur around *débris* in the alveoli.

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Cystic Disease of the Lungs

Cysts in the lung may be due to congenital malformation or to other causes, but the differentiation of these types may be difficult or often impossible. Congenital cysts may be associated with infection ; on the other hand, inflammatory processes which give rise to cysts may subside, leaving only a diffuse fibrosis. An additional difficulty is that spaces in the lung caused by abscess formation may subsequently become lined with ciliated or squamous epithelium and be mistaken for developmental cysts (Pryce, 1948).

Oswald and Parkinson (1949) described 16 cases in which there were thin-walled cysts distributed throughout both lungs. The cysts were of varying size up to 1 cm. in diameter and the condition was described as " honey-combed " lungs. In 6 cases the lesions were associated with some other condition, namely, xanthomatosis, biliary cirrhosis, tuberous sclerosis, or pituitary disease ; in the remaining 10 cases an inflammatory cause was suspected. The histology of 9 of these was examined in detail (Cunningham and Parkinson, 1950) and 6 were thought to represent different stages of a common process. In relation to the cystic spaces there was a granulomatous exudate consisting of large numbers of histiocytes, together with lymphocytes, plasma cells, giant cells and eosinophiles. Some of the histiocytes had a foamy appearance, but the authors had no fresh tissue for frozen sections, so the question of the presence of lipoid was not definitely decided. In some of the cysts the granulomatous tissue formed the whole wall, while in others there was a lining of cuboidal epithelium. The cuboidal cells fused to form giant cells, some of which desquamated into the cystic spaces (this description is reminiscent of that of Hecht of the giant-cell pneumonia of infants). In some of the cases there was diffuse interstitial fibrosis which was regarded as a later stage of the process. Two of the cases had a similar granulomatous condition in the liver and 1 had transient diabetes insipidus. The process was considered as not due to tuberculosis or syphilis, nor to pyogenic or virus pneumonia, and the changes were unlike those of the diffuse interstitial fibrosis described by Hamman and Rich (1944). The condition was considered not to be sarcoidosis, polyarteritis, or beryllium poisoning, but rather as belonging to the group of disorders included under the heading of " eosinophilic xanthomatous granuloma." It has previously been shown that

" honey-combed " lungs are found in this latter condition and that fibrosis occurs in the chronic phase. Cunningham and Parkinson say alternatively that the granuloma may be a non-specific response to a number of causes, but they are satisfied that the granulomatous infiltration, whatever its cause, is responsible for the cystic changes which arise as a result of weakening of the walls of the smaller bronchioles.

Oswald and Parkinson consider that the mechanisms leading to the formation of the cysts resemble those responsible for focal emphysema in pneumoconiosis. This is unlikely since the structure of the cystic spaces in focal emphysema is different from those in " honey-combed " lungs. In pneumoconiosis strands of tissue run across the spaces to central dust foci, but there is no such structural arrangement in the " honey-combed " lungs of the above authors. " Honey-combed " lung occurring in coal-miners is distinguishable from severe focal emphysema.

In generalized scleroderma, Church and Ellis (1950) have described 2 cases of pulmonary cysts. Clinically the changes are similar to those described in the " honey-combed " lung of Oswald and Parkinson. In one of the cases iodized oil introduced into the bronchial tree did not enter the cystic cavities, suggesting that there was obstruction of the bronchioles.

Changes in the framework of the lung from many causes can thus apparently lead to widespread cyst formation, and it has been suggested that bronchiolar obstruction and weakening of the bronchial walls are the determining factors in the development of the cysts in these various diseases.

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Intralobar Sequestration of Lung

This condition may be mentioned here as it is sometimes accompanied by cystic change. It is associated with an abnormal artery from the aorta to the lower part of the lung, and, although recognized only rarely in the past, has been amply described by Pryce (1946), who has also offered an explanation of the condition on embryological grounds. In some instances the artery supplies

normally-functioning lung, but in others it vascularizes a sequestrated portion of lung whose bronchi do not communicate with the rest of the bronchial tree. Pryce suggests that the abnormal blood supply is the fundamental defect and that in some instances the abnormal artery " captured " a lung bud, which then develops separately from the rest of the lung. The different grades of abnormality which may arise are discussed by Pryce and his co-workers (1947–48). This abnormality is very likely to be followed by sepsis and to be diagnosed as an ordinary instance of bronchiectasis, but its true nature is being recognized more frequently by thoracic surgeons, the abnormal artery, lying in the line of the pulmonary ligament, sometimes attracting attention at operation. An interesting feature is that the artery, although arising directly from the aorta in the region of the diaphragm, is of elastic type like the pulmonary artery and not muscular like bronchial, renal or coronary arteries.

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THE PNEUMOCONIOSES

In 1934, according to Kettle, the view was generally held that pneumoconiosis and silicosis were synonymous, and that the only dusts capable of producing fibrosis of the lungs were those containing a considerable proportion of some form of silicon. Since that time new forms of pneumoconiosis have appeared ; some have occurred in new industries which deal with beryllium and aluminium, while another has resulted from the inhalation of the dust of sugar-cane (bagasse). These newly-recognized forms of dust disease have not affected many individuals. It is the high incidence of pneumoconiosis in the coal industry, not only in Great Britain, but also in Australia, Belgium, France, Germany and elsewhere that has been one of the main causes for concern in recent years. The relationship of coalminers' pneumoconiosis to classical silicosis has been the subject of much investigation, both in respect of morbid anatomy and pathogenesis, and there is still debate as to whether the dust in coalminers' lungs acts mainly in a mechanical or mainly in a chemical manner.

Pneumoconiosis similar to that in coal workers occurs in graphite workers, and the pathology of this disease was described

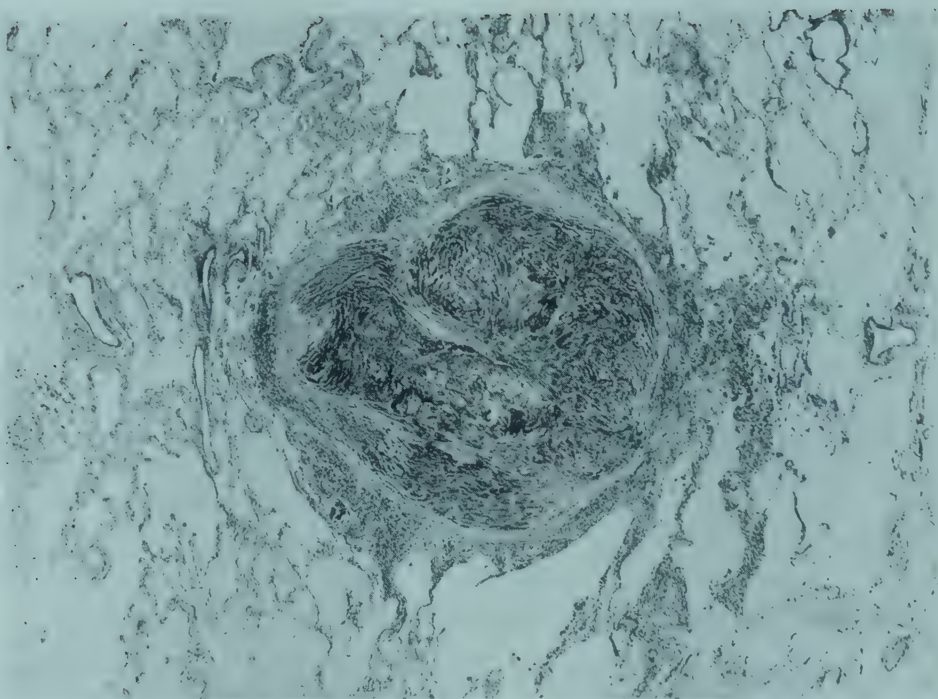

FIG. 9/15. Silicotic nodule. (Stained aniline blue. × 20.)

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for the first time in this country in 1949. Recent research in silicosis has included further studies of the interaction of various components in mixed dusts, with special reference to the neutralizing action of aluminium on the toxicity of silica. The results explain some of the variations in the pathogenicity of siliceous dusts.

Silicosis

The basic lesion of classical silicosis is the familiar silicotic nodule in which fibrous tissue is laid down, usually in a concentric arrangement (Fig. 9/15). Nodules of this type are produced in man and experimental animals by exposure to dusty atmospheres rich in free silica (silicon dioxide). According to early workers (Gye and Purdy, 1922 ; Gye and Kettle, 1922) the toxic action is explained by the silica going into solution and acting as a cell poison. Kettle (1932) showed that the silica could be rendered insoluble and non-toxic by coating it with iron ; similar results have been obtained with aluminium (Denny *et al.*, 1939). To produce the neutralizing effect the two substances must be brought into direct contact. This results in the formation of a layer of aluminium hydroxide on the silica particles, making the latter insoluble. The effects of the inhalation of silica alone were compared with those of the inhalation of silica mixed with aluminium. The mixture did not produce silicosis in animals, even when administered in high concentration for several hours a day over a period of many months, while the disease developed in animals receiving the silica dust alone. Similar protection was obtained when silica and aluminium were given at separate times during the day, showing that aluminium when inhaled could neutralize silica already in the lung. This protective effect was not obtained when a mixture of silica and aluminium was injected in a single dose into the trachea (Belt and King, 1943). This discrepancy has been explained (King *et al.*, 1950) as being due to the aluminium being absorbed from the lungs, leaving the silica free to go slowly into solution and cause fibrosis, and it is suggested that aluminium can prevent or retard silicosis only if it is repeatedly supplied to the lung. The administration of quartz mixed with 2 per cent. powdered aluminium daily in the atmosphere confirmed the earlier observation (Denny *et al.*, 1939) that there was marked retardation of the development of silicosis. Aluminium has been given by inhalation in the treatment of established silicosis in man, but so far there has been no conclusive

objective evidence of improvement in the pathological changes in the lungs. Subjective improvement has been obtained, but further enquiry is necessary before the reasons for this can be assessed.

In a critical study of the theories of silicosis, King (1947) points out that according to the modern " chemical " theory, the toxic substance is silicic acid, and it might therefore be supposed that the pathogenicity of any stone dust would bear a direct relation to the rate at which it will release silicic acid into solution. In general, he found this to be true. Quartz and flint, which dissolve to the extent of 10 mg. of silica per 100 c.c. of blood and plasma, are the most pathogenic, while shale and mica are less soluble and less pathogenic. There are, however, exceptions to this rule. Certain sandstones with a low silica solubility have a high pathogenicity, while olivine, a magnesium silicate of intermediate solubility, apparently causes no pulmonary disease in man and no fibrosis in animal lungs. King emphasizes that in mixed dusts some of the constituents may depress the solubility of the free silica component, thus shale dust markedly depresses the solubility of quartz dusts. The interaction of the different components would appear to explain why some dusts have a lower degree of toxicity than might have been expected from their total silica content. Variation in solubility is, however, not a complete explanation of the relative toxicity of siliceous dusts, and King suggests that liberation of a toxic substance from silica in the body may be different from that which occurs *in vitro*. He speculates on the possibility that within cells silicic acid may be released in a special toxic or " nascent " form. Others have attempted to interpret the pathological reactions to silica in terms of surface activity of the particles, and have inferred that newly-formed particles are more active in producing disease than are older ones. Policard (1947) suggests that old dust such as sand in the natural state has become less pathogenic due to weathering ; he considers that this is due to the loss of bases and the intake of water, whereby the particles lose their effect on living tissues. King (1945) observed that dust kept in a laboratory in a dry state had retained its pathogenicity after many years, but particles repeatedly leached with water lost solubility, and this phenomenon may be concerned in the weathering of dust. On the other hand, Gardner (1938), who raised many objections to the solubility theories of silicosis, noted that a sterile suspension of silica in physiological salt solution kept in a refrigerator for many months

neither increased nor decreased in capacity to excite reaction in the tissues. Thus, while there is some evidence that newly-formed particles of silica are the more pathogenic, recent formation of particles is not a prerequisite for toxicity.

The size of the particles concerned in the production of silicosis is important. Those which enter the lung are mostly below 10μ and the majority below 5μ. King (1947) has shown that in Ringer's solution there is increased solubility with reduction of particle size below 5μ, and Gardner (1939) considers that the toxic action of quartz in chronic lesions is limited to particles of 3μ or less. Interest hitherto has been mainly in particles of 1–5μ, but more recently attention has been directed to particles of submicroscopic size. Very finely-divided silica, such as the so-called "20 angstrom silica" ($0{\cdot}002\mu$), has a general toxic action, but does not cause pneumoconiosis in animals. King (1947) gave intratracheal injections of these very minute particles in doses comparable with the weight required to produce silicotic nodules when larger particles are used. The preparations of the finer particles, however, caused death within a few hours. The minute particles rapidly form a colloidal solution of silicic acid which is absorbed into the circulation producing a general toxic effect. Animals receiving smaller doses survived for several months, but silicosis did not develop in them. It seems that the particles of silica which produce fibrosis are within a limited size range, and there is suggestive, but by no means conclusive, evidence that fibrogenic activity is determined by the rate at which silica goes into solution.

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Pneumoconiosis due to Silicates

Fibrous Silicates: Asbestos, Talc. The question as to whether silicates are toxic when inhaled has been very much debated since Jones (1933) put forward the view that sericite, a potassium aluminium silicate, is the cause of much industrial silicosis. Of recent years, however, his view has received very little support. Certain silicates are noxious, and the chief of these is the group of fibrous mineral silicates known as asbestos. There are two views regarding the mode of development of asbestosis. According to the one, the action of the dust is chemical; the other view supposes that the asbestos fibres act mechanically. Experiments carried out at the Saranac laboratories (Trudeau Annual Report, 1948) do not support the chemical theory, since aluminium hydroxide which neutralizes the toxic action of quartz does not prevent asbestosis. Moreover, asbestos fibres shorter than 20μ were found to be relatively innocuous, whereas if the action were chemical an increase in potency would have been expected with reduction in particle size below 5μ, as in the case of silica. A comparison of the effect of asbestos fibres 15μ long with those $2{\cdot}5\mu$ long (King *et al.*, 1946) showed that rabbits receiving the long fibres developed a nodular reticulin formation comparable with experimental silicotic nodules; animals receiving the short fibres developed a diffuse interstitial reticulin formation. The localization of the asbestos lesions appeared to depend on the size of the fibres; with long fibres, too big for removal by phagocytes, the reaction resulted in the formation of connective tissue within the alveoli; with shorter fibres capable of being taken into the alveolar walls there was diffuse interstitial fibrosis. These workers suggest that if the fibres were shorter than $2{\cdot}5\mu$ they would be completely removed from the alveolar walls and fail to produce fibrosis. Johnstone (1948) concluded from clinical investigation that workers exposed to high concentrations of extremely fine asbestos dust did not suffer from asbestosis. The Saranac workers conclude that asbestosis is caused by mechanical irritation from the asbestos fibres during the movements of respiration, and that this peculiarity is related to the flexibility of the fibres not possessed by other foreign bodies. The view that movement of the lungs is a factor is supported by the absence of reaction to asbestos in organs other than the lung (Gardner, 1938). Wyers (1949) points out that if the fibrous character is absent, as in the case

of serpentine, the chemical equivalent of asbestos, the dust is inert.

The association of carcinoma with asbestosis has been mentioned above, but more details may be given here. An analysis of 235 cases of asbestosis is given in the 1947 report of the British Chief Inspector of Factories. Cancer of the lung or pleura occurred in 31 (13·2 per cent., average age 52·1 years). These figures were contrasted with the much lower incidence of cancer of the lung in 6,884 cases of silicosis, of whom 1·32 per cent. had carcinoma (average age 59·4 years). A high incidence of tuberculosis was also associated with asbestosis, and the Inspector of Factories found 72 instances in 130 cases of asbestosis.

Talc. Numerous cases of pneumoconiosis due to talc (hydrated magnesium silicate) have been described in different parts of the world, and the first of this type in Britain, proved by autopsy, was reported in 1949 (McLaughlin *et al.*). Throughout the lungs there were grey nodules which had coalesced in some places, especially in the lower lobes. Microscopically the nodules showed whorling, but this had not the typical appearance of silicosis. Within the nodules were fibre-like structures up to 40μ in length arranged singly and in clumps. Some were very similar to asbestos bodies, and the appearances suggested that talc pneumoconiosis and asbestosis are similar diseases and that the former may be caused only by the fibrous varieties of talc. Asbestos is more actively fibrogenic than talc, and this might be due to the higher proportion of fibres in asbestos. In New York State disabling pneumoconiosis occurred in 14·5 per cent. of the workers in the talc mining and milling industry (Siegal *et al.*, 1943). The dust contained fine, straight, needle-like fibres. The talc fibrosis was associated with an increased susceptibility to tuberculosis.

Glass Wool. Glass in the form of fine fibres is being used in increasing quantities for heat insulation, and during its preparation and use the atmosphere may shimmer as a result of floating fibres. No harm appears to result, however, from exposure to this dust, since the fibres do not remain suspended in appreciable concentration owing to the high electrostatic charge on them. Gardner (1942) considers that the material has not the properties required to produce fibrosis.

Non-fibrous Silicates : Fuller's Earth ; Mica. Fuller's earth, composed mainly of a silicate of aluminium, is found in Surrey, where it is quarried, dried and crushed. Middleton (1940) has given an account of the radiological and pathological changes

due to this dust. There is pulmonary fibrosis, but it is less dense than the hard nodular type seen in classical silicosis. One of the Surrey workers emigrated to Canada and about fifty years later died from pneumonia following radium treatment of a cancer of the lip. At autopsy Tonning (1949) found a pneumoconiosis corresponding to that previously described in workers exposed to fuller's earth ; the lesions still contained an abundance of the mineral.

Mica. Cases of pneumoconiosis have been described in men exposed to a mica dust containing almost no free silica (Dreesen *et al.*, 1940) ; the signs and symptoms were said to resemble those of silicosis.

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Coal-Workers' Pneumoconiosis

The small percentage of coal-miners who develop classical silicosis are men engaged in drilling rock, cutting through strata which separate the coal seams. Some workers may engage entirely on this type of work, and their lungs, as for instance those working in the sandstone strata in the east of South Wales, may show silicosis indistinguishable from that found in gold-miners, tin-miners and stonemasons. On the other hand, the men who work on the coal seams, cutting the coal with hand picks or by machinery, and those engaged in loading coal, present a pneumoconiosis distinguishable from silicosis of the classical type. As with classical silicosis, there appears to be two forms of the disease : one, simple pneumoconiosis due to action of dust alone ; the other, infected pneumoconiosis due to the combined action of dust and tuberculosis (Gough, 1949).

FIG. 9/16. Coal nodule with focal emphysema. × 20.

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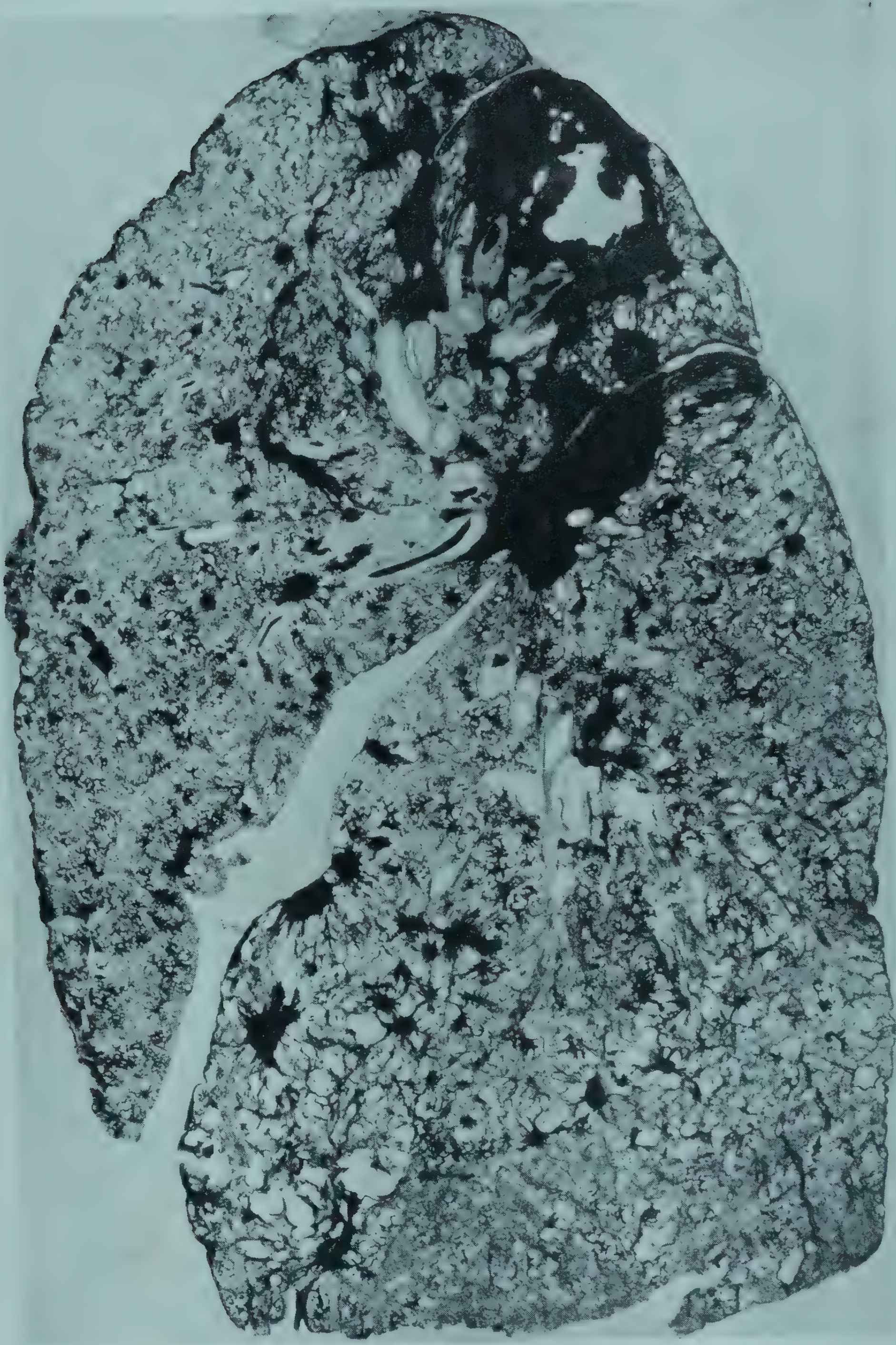

Fig. 9/17. Infective variety of coal-workers' pneumoconiosis. Sagittal thin section of left lung. × approx. ½.

Simple Pneumoconiosis. In the simple form the dust is found throughout the lungs in the form of black foci measuring up to about 5 mm. in diameter. The dust collects around the small bronchioles and their accompanying arteries having been brought there from the alveoli by phagocytes. For the most part the dust remains within these cells, the general shape of which is preserved, although the nuclei are obscured. Reticulin fibres develop in the foci of dust (Belt and Ferris, 1942). Fibrosis may not proceed beyond this stage or there may be the development of collagen. The latter does not develop to the same extent as in classical silicosis nor does it have the concentric disposition, but runs irregularly or radially. The foci have a crenated edge with the processes extending into the unaffected tissues. In and around the coal foci the air-spaces become dilated, giving a characteristic appearance described as focal emphysema (Fig. 9/16). This emphysema has also been described in classical silicosis, but it is very much more severe in the coal worker, and in the latter the emphysematous spaces may enlarge and become confluent (Gough, 1947, 1949). The focal emphysema appears to be due to some mechanical disturbance within the secondary lobules of the lung, as in these units the emphysema starts around the bronchioles and extends outward towards the interlobular septa. In focal emphysema there are no bullæ projecting from the surface of the lung, so that the condition can be distinguished from ordinary bullous emphysema.

Williams (1944) observed that there was interference with the lumen of the terminal bronchioles, causing partial obstruction with trapping of air and consequent development of emphysema. Heppleston (1947) considers that the development of focal emphysema in coal workers can be explained by the accumulated dust acting mechanically, the foci of coal dust interfering with the function of the respiratory bronchioles. He also considers that shrinkage of the dust foci contributes to the development of surrounding emphysema.

Many investigators (including King and Nagelschmidt, 1945) regard coal-miners' pneumoconiosis as a form of silicosis, and they contend that the small amount of quartz in the dust is the pathogenic agent, although their chemical analysis of coal-miners' lungs does not give conclusive support to this view. King (1945) found that the dust from the Welsh coal mines has a low silica solubility, and Hicks and Nagelschmidt (1943) failed to find any marked relationship between the silica content of the

dust in the various Welsh coal mines and the incidence of the disease. They suggest that the latter may be related to the rank of coal in the different mines ; by " rank " is meant the amount of volatile material present. Anthracite coal with a low volatile content is of high rank ; bituminous coal with a high volatile content is of low rank ; intermediate are the steam coals. In Wales the incidence of disease is higher in the anthracite and steam coal areas than in the bituminous areas.

The opposing theories of mechanical and chemical action of the dust in coal miners' lungs may be reconciled by accepting that each is in part correct, as is suggested by the following experiments. King and his co-workers (1948), treating rats by intra-tracheal injections of coals mixed with quartz, found that the mixtures produced more fibrosis than quartz alone, and they conclude that a small amount of quartz in the presence of a large amount of coal will bring about greater pathological results than the probable sum of the two components used separately. No difference was found between bituminous and anthracite coals, either when given alone or when mixed with quartz, and it is suggested that perhaps the coal dust blocks the lymphatic channels so that the quartz remains longer in the lung. These results support the conclusion that the harmful effect of coal is not restricted to its silica content. The practical result of such a view is that all dusts in the coal mine should be regarded as harmful and should be suppressed, and indeed dust suppression methods are being directed to that end. Pneumoconiosis identical with that in coal-miners is seen in coal-trimmers, who load coal into ships (Gough, 1940). The finding of pneumoconiosis in these men shows conclusively that the dust of commercial coal is noxious. In the past it has been argued that the respiratory disease in coal-miners may be due to the fumes from the explosives used in getting the coal, or to severe chilling when leaving the working place on the long journeys underground against the incoming air. The occurrence of an identical form of the disease in men loading coal into ships shows that cold and fumes can be at the most only contributory factors and that the essential cause is the inhalation of dust.

Infective Variety of Coal Workers' Pneumoconiosis. The condition described above as " simple pneumoconiosis " seems to be due to the action of dust alone, but superimposed upon it there is frequently a fibrosis which appears to be due to the combined action of tuberculosis and dust (Fig. 9/17). This fibrosis occurs in the form of circumscribed masses most frequently in

the upper and posterior parts of the lungs, sometimes unilateral, usually bilateral. The fibrous masses are black and may be several inches in diameter, and are firm and rubbery in consistency. The centres of the masses often contain black inky fluid in which are crystals of cholesterol ; in some cases the fluid is expectorated, leaving ragged cavities. These cavities appear to be formed by necrosis due to obliterative endarteritis.

In about 40 per cent. of cases of massive fibrosis tubercle bacilli can be demonstrated in the lesions post-mortem by culture or animal inoculation, and in those cases where no bacilli are found it is assumed that the infection has died out. In the past, tuberculosis has been regarded as of low incidence in coal workers, but the form of tuberculous reaction which occurs does not usually give rise to caseation or to the usual symptoms of open tuberculosis. There is greater formation of fibrous tissue, the progress is very slow and the ill-effects are principally on the pulmonary circulation, causing very marked right heart hypertrophy and death due to congestive cardiac failure. In a smaller percentage of cases the tuberculosis spreads more acutely, and in these there is caseation and bronchopneumonic spread of the infection. Another cause of death is pulmonary arterial thrombosis. This commences in a branch of the artery in relation to an area of massive fibrosis and the thrombus is propagated towards the hilum of the lung.

In summarizing the outstanding differences between coal-workers' pneumoconiosis and classical silicosis emphasis is placed first on the more severe focal emphysema in the coal-workers' lung, and secondly on the somewhat different effects of tuberculosis in the two conditions. In classical silicosis the open type of tuberculosis is a common terminal event, although heart failure from massive fibrosis also occurs ; in coal-workers, massive fibrosis leading to heart failure is the usual occurrence, whereas open tuberculosis is a less common complication.

Graphite Pneumoconiosis. Natural graphite is composed of crystalline carbon with up to 10 per cent. free silica and other minerals. In the past graphite, like coal, has been regarded as innocuous, but two recent papers (Gloyne *et al.*, 1949 ; Harding *et al.*, 1949) have described graphite pneumoconiosis in Britain ; the disease is similar to that met with in coal-workers.

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Pulmonary Disease in Beryllium Workers

Before 1930 little industrial use was made of beryllium or its compounds, but their valuable properties have since been recognized and employed in rapidly increasing amounts. In 1931 only 2 tons of beryllium ore was used in the U.S.A., but in 1946 consumption had increased to 1,400 tons. The source of beryllium is the ore beryl (beryllium aluminium silicate) and most of the metal extracted is used as an alloy with copper in order to strengthen the latter ; some is used as an ingredient in the powder lining fluorescent light tubes. Beryllium is also used in atomic piles, so that further increases in the amounts employed for this and other purposes are to be expected. Workers exposed to beryllium and its compounds have shown diseases of the skin, the eyes, and the respiratory organs. Two forms of pulmonary disease occur—a chronic granulomatous inflammation of the lungs, and an acute pneumonitis. Most of the information concerning these conditions has come from the U.S.A., for only very few cases have been reported in Great Britain, and the 1947 Report of the Chief Inspector of Factories does not mention beryllium as an industrial hazard. The first case reported in Britain (Agate, 1948) occurred in a man who had been working on the development of fluorescent lamps, and this is the only case mentioned by the Chief Inspector of Factories in his 1948 Report.

Pulmonary Granulomatosis in Beryllium Workers. In 1941 in Massachussetts, cases of pulmonary disease of an unusual type appeared in several workers in a plant manufacturing fluorescent lamps. The fluorescent substance (phosphor) used was zinc manganese beryllium silicate. Inhalation of this substance was probably the cause of the disease. A similar condition was discovered in Connecticut in men making beryllium copper alloys (Jackson, 1950) ; 5 cases, 2 of them fatal, have been

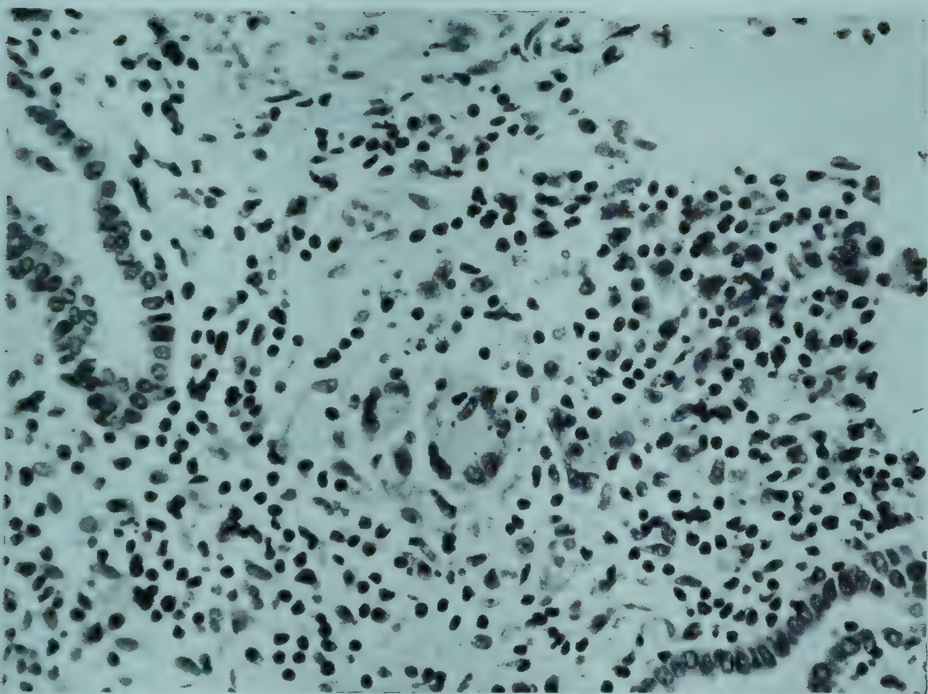

FIG. 9/18. Beryllium granuloma in lung. × 280.

[To face page 228.

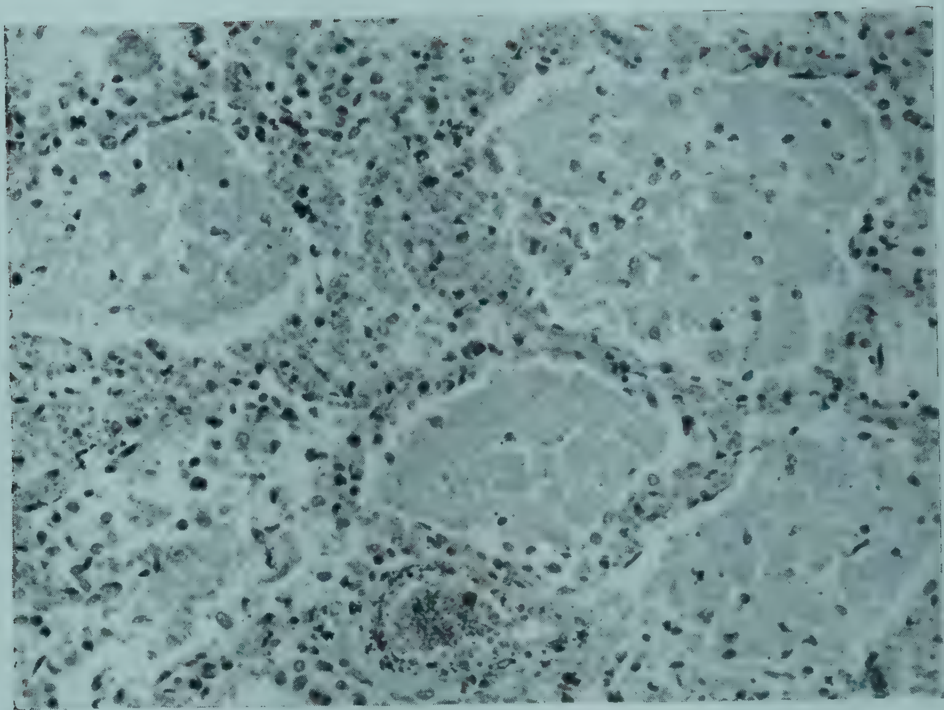

FIG. 9/19. Acute beryllium pneumonitis. × 200.

reported in young women employed in radio valve factories (Slavine, 1949); and accounts have been published of cases occurring in the fluorescent lamp industry (Hardy and Tabershaw, 1946; Hardy, 1950). The symptoms began gradually with increasing fatigue and loss of weight, followed by dyspnœa on exertion. Some of the patients had cough and extremely shallow breathing and later developed orthopnœa. Cyanosis was frequently present, and in a few there was enlargement of the lymph glands, liver and spleen. Several died after an illness lasting approximately two years. Hardy described the condition under the name of "delayed chemical pneumonitis" as there was often a latent period, sometimes of several years, between exposure to the toxic substance and the development of symptoms. Post-mortem examination showed a diffuse inflammation of the lungs with infiltration with lymphocytes, plasma cells and large mononuclear cells, together with a varying number of multinucleated giant cells. Many of the air spaces were obliterated and others were lined with cuboidal epithelium (Fig. 9/18). Although the inflammation was for the most part diffuse, similar changes were found in discrete nodular foci. The same kind of granuloma was present in the hilar lymph nodes and in some cases in the liver. The histological appearances simulated Boeck's sarcoidosis, even to the extent of having concoidal bodies, but Hardy considers that the condition is distinguishable clinically from Boeck's disease. Unlike the latter, beryllium granuloma is a severe condition with a poor prognosis. It is only occasionally accompanied by an increase in serum globulin and there is no involvement of the bones. Moreover, there is an occupational explanation of the beryllium disease.

Acute Pneumonitis in Beryllium Workers. In Ohio, in plants engaged in extracting beryllium from its ore and in the manufacture of various beryllium compounds and alloys, a number of employees have shown changes in the skin and in the lungs (DeNardi, 1950; Van Ordstrand, 1950) believed to be due to the action of beryllium. A similar condition has been described by Italian, German and Russian workers in the period 1933–40, and a summary of the literature is given by Hardy in her article published in 1950. The American cases have been described under the name "Acute Pneumonitis," although DeNardi points out that while some of them are fulminating, others are insidious in their onset. In Van Ordstrand's cases the pneumonitis occurred only in those workers actually extracting the beryllium, while

others in the plant were not affected. Symptoms recurred in affected workers if they returned to work involving exposure to beryllium. Mild cases showed only a rhinitis, but in more severe ones there was inflammation of the lower air passages and of the lungs. The majority of patients recovered completely, but some died. Post-mortem the lungs showed swelling of the alveolar walls and albuminous exudate in which there were mononuclear cells (Fig. 9/19); there were very few polymorphonuclear leucocytes, but plasma cells were often abundant. The only other notable finding in cases of acute beryllium pneumonitis was *cor pulmonale*. Dutra (1948) found no sharp distinction between the acute and chronic forms of beryllium pulmonary disease and he described grades of transition between the two types.

Neighbour Cases. One remarkable and baffling feature in the story of beryllium disease has been its appearance in individuals not employed in the industry, but living in the neighbourhood of beryllium factories. The evidence indicates that the source of the poison was in some cases airborne dust from the factories, while in other instances workers living in the same houses may have brought home the poison on their clothes. Beryllium was found post-mortem in the lungs of these so-called "neighbour" cases.

Animal Experiments. The disease as found in man has been reproduced fairly closely in experimental animals. Policard (1948) produced both the pneumonic and granulomatous forms in experimental animals by introducing beryllium compounds into the lungs in single doses. Beryllium sodium fluoride produced violent pulmonary œdema and death of the animals within twenty-four hours. Beryllium oxide produced pneumonia with histocyte reaction followed by nodular granulomata which were histologically similar to Boeck's sarcoid. Denz (1949) has described a histochemical method for detecting beryllium and has shown that the pathological lesions are associated with focal concentrations of beryllium. Scott (1948) found that inhalation of beryllium sulphate dust produced inflammation of the terminal bronchi. The exudate contained very few leucocytes; invasion of the exudate by fibroblasts was occasionally seen, but fibrosis did not develop. LaBelle and Cucci (1948) noted that the response was different for the soluble and insoluble beryllium compounds. Soluble ones caused acute lesions, but insoluble ones caused chronic effects. Vorwald (1950) also produced chronic granulomatous reactions with insoluble beryllium compounds but not

reproducing in any sense the granulomatous lesions of human cases. Lloyd Davies and Harding (1950) obtained granulomata in rats' lungs by introducing beryllium oxide into the trachea. They found that larger areas of granuloma occurred when the action of beryllium was combined with that of manganese, and they suggest that the dusts that have produced chronic granulomata in man are mixtures containing other elements besides beryllium. In rabbits beryllium causes sarcoma of bones, but no human cases of tumours due to the poison have yet been recorded.

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Pneumoconiosis due to Aluminium

Aluminium has been found to reduce the toxic action of silica, but, on the other hand, a pneumoconiosis has been ascribed in Germany to the dust of powdered metallic aluminium (Goralewski, 1943). Among the clinical features was the development of spontaneous pneumothorax in several cases. Collagenous fibrosis was found post-mortem and the dust particles present in the lesions could be distinguished from carbon by their jagged edges.

In the lungs of experimental animals aluminium dust alone produces pneumonia, but no fibrosis, but when the animals are exposed to cold or given pneumococcal infection severe fibrosis develops and the appearances are similar to the human disease

(Jötten and Eickhoff, 1943). No pneumoconiosis was found in grinders of duralumin (an alloy containing 95 per cent. aluminium) in Britain (Hunter *et al.*, 1944), but the Chief Inspector of Factories, in his report for 1945, mentions that at least one death in this country has been ascribed to the inhalation of particles of aluminium. The more numerous cases in German workers may have been due to the omission of lubricants in the stamping process of making aluminium powder, or perhaps to the increase in the amount of dust in the atmosphere in consequence of unsatisfactory ventilation of workrooms resulting from the war-time blackout. The addition of lubricants in animal experiments has been found to diminish the ill-effects of aluminium dust (Marwyck and Eickhoff, 1950), and these authors also note that with the return to peace-time conditions hardly any new cases of aluminium pneumoconiosis are arising in German industry. Examination of material from the German cases shows that there was undoubtedly a pneumoconiosis, so that aluminium dust must be regarded as a toxic substance under certain conditions of exposure.

Pneumoconiosis in Corundum Workers (Shaver's Disease). In connection with aluminium another newly-recognized form of pneumoconiosis may be considered. This has been described in Canada (Shaver and Riddell, 1947 ; Shaver, 1948) as occurring in men exposed to the fumes arising in the process of making the abrasive " corundum " (aluminium oxide) by heating bauxite (ore containing approximately 80 per cent. aluminium oxide and 5–7 per cent. silica) with iron and coke. Silicotic nodules did not develop, but there was diffuse fibrosis and the formation of emphysematous bullæ which fused together to form larger cavities—rupture of these often gave rise to pneumothorax. The precise ætiology of the lung fibrosis is uncertain as the noxious atmosphere contains high concentrations of fumes of both aluminium and silica in a very fine state of division. Pratt (1950) found that the fumes contained 32·3 per cent. silicon dioxide and 56 per cent. aluminium oxide, the silica being almost entirely in amorphous form. Hatch (1950) reports that the particles are 0·02–0·5μ, and that particles of such size have in the past been regarded as of little significance in the development of pulmonary fibrosis (it has been mentioned above that still smaller particles, " 20 Angstrom silica " have a general toxic action, but do not cause pneumoconiosis in experimental animals). If the disease is to be regarded as an unusual form of silicosis, the question may

be asked why the aluminium in the fumes does not neutralize the action of the silica. Irwin (1950) states that in order to render silica insoluble by means of alumina the latter must be of a type which readily takes up water and becomes adsorbed on the silica particles. The alumina in the fumes is not of that type. Whatever be the noxious element in the fumes causing the disease in corundum workers the reaction in the lung is very different anatomically from classical silicosis.

Pulmonary Disease due to Nickel and Cadmium Dust. Friberg (1948) has described emphysema in workers exposed to the dust of cadmium and nickel in a plant making storage batteries. The changes occurred only after many years of exposure. The precise cause of the condition is not known, but it is believed that cadmium is the toxic component.

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Non-pathogenic Inorganic Dusts

Experience has shown that certain dusts formerly regarded as harmless have subsequently been found to be noxious ; a notable example is the dust of commercial coal, and caution is therefore necessary in regarding any dust as harmless. Nevertheless, certain dusts appear to be without ill-effect, although these are important because their accumulation in the lungs may lead to radiographic appearances which closely simulate serious forms of pneumoconiosis. For instance, iron oxide particles are inhaled by electric arc welders and cause an X-ray appearance resembling silicosis, but these men maintain good health and are fit to work, and there is no increased liability to tuberculosis (Doig and

McLaughlin, 1936). The first account of a post-mortem examination of the lungs of an arc welder (Enzer and Sander, 1938) reported the presence of iron in the perivascular and peribronchial tissues and in the lung septa. The authors remarked that the fumes produced in arc welding consist of 99 per cent. iron oxide, and it is the accumulation of this which causes the X-ray nodulation. Doig and McLaughlin (1948) re-examined arc welders with X-ray changes in their lungs over a period of years, and found that in two the X-ray appearance changed for the better and in one the picture had returned to normal some years after giving up welding, indicating that iron oxide can be absorbed from the lung.

Silver polishing is another occupation in which iron oxide is inhaled. Post-mortem examination of the lungs of a silver polisher, whose work consisted of using rouge (Fe_2O_3) as an abrasive, showed appearances similar to those of electric arc welders (McLaughlin *et al.*, 1945). Barrie and Harding (1947) described the post-mortem findings in three silver polishers. Iron oxide and silver was found in the lung ; the former was in phagocytic cells along the course of the lymphatics and had produced no interstitial fibrosis. The silver dust had an affinity for the elastic tissue of the alveolar walls and the small pulmonary vessels. In 2 cases there was marked emphysema, but the authors were not prepared to associate this with the silver impregnation of the alveolar walls. One of the men had complained of cough for several years, but the others had given no history of respiratory disability. A condition similar to that in arc welders has been described (Pendergrass and Pryde, 1948) in a man inhaling tin oxide ; in this case there was little fibrosis and no true nodulation. Bartak and Tomecka (1948) also considered that tin oxide, although producing radiographic changes, does not produce ill-effects clinically. Barium is considered to be inert in the lungs, but accumulation will produce marked radio-opacity.

Carborundum (silicon carbide) has been regarded as harmless, since the classical experiments of Gardner (1923) showed that it was not toxic to experimental animals. His work was one of the most important pieces of evidence in disposing of the theory that it was the hardness or sharpness of particles that causes damage in the lung, since carborundum is exceedingly hard. It would be ironical if it was now found that carborundum produces pneumoconiosis in man. Radiographic evidence of pneumoconiosis in men exposed exclusively to carborundum dust has been

produced (Bruusgaard, 1948), and there is further evidence that this dust may produce pneumoconiosis (Smith and Perina, 1949). These authors suggest that the question of pathogenicity of this material will need to be reconsidered and further investigation, including post-mortem studies, will be necessary before the position can be clarified. It may be mentioned, however, that Gardner (1938) found in experimental animals that carborundum, together with tuberculous infection, could produce a fibrotic reaction, and this may perhaps explain the human cases.

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Pathogenic Organic Dusts

Many dusts of vegetable origin are known to produce respiratory symptoms, but there are no distinctive changes in the lungs similar to those produced by mineral dusts. Cotton workers, for instance, develop dyspnœa, due apparently to the inhalation of some ingredient of the dust in the workrooms, but the changes in the lungs are those of bronchitis and emphysema indistinguishable from the ordinary forms of those diseases (Dunn and Sheehan, 1932). Other vegetable dusts which have been incriminated include those of grain, flax, jute and tobacco. These vegetable dusts may have a common mode of action, which appears to depend at least in part on allergic reaction.

Bagassosis. One recently recognized form of pneumoconiosis is ascribed to the dust of sugar-cane (bagasse). An account of the condition, including 1 fatal case, has been reported (Hunter and Perry, 1946); the men affected were engaged in breaking up bales of cane imported into Britain. Most of the sugar had

been extracted from the cane before export, although a small residue formed a good culture medium, and when the cane reached Britain it was heavily contaminated with organisms, including moulds. Perry (1948) found that the bronchioles become filled with vegetable dust which swells under the influence of bronchial secretions, blocks the bronchioles and gives rise to small areas of collapse. When these areas become infected, acute bronchiolitis and pneumonia result. In some cases the condition does not resolve and there develops a fibrosis of the lung with cough and sputum and much shortness of breath, together with radiographic changes showing thick bands of fibrous tissue traversing the lung field so as to simulate cavities. In 1 case necropsy revealed chronic bronchiolitis and bronchiectasis. Fungi may play an important *rôle* in breaking down the fibres into a very fine vegetable dust and may possibly even render this toxic. Aspergillus is present in all specimens of bagasse dust, but Perry considers that these organisms take no part in causing the disease. On the other hand, administration of bagasse to rabbits (Gerstl *et al.*, 1949) produced pneumonic lesions, from which aspergillus was isolated. Bagasse which had been autoclaved caused only a foreign body giant cell reaction and slight fibrosis, and these workers conclude that the pneumonic changes are due to the micro-organisms present on the bagasse. Bagassosis resembles the condition of " Farmer's Lung " and of " Broken Wind " in horses, caused by the inhalation of the dust of mouldy hay.

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A LARGE SECTION TECHNIQUE FOR THE STUDY OF LUNG PATHOLOGY

Large sections have previously been used in pathology but a new technique introduced by Gough and Wentworth (1948; 1949) has certain advantages over earlier methods in that the natural colours are preserved, and the sections are mounted on paper. With these translucent preparations certain features of lung pathology can be demonstrated more clearly than by other means. The original purpose of the method was to produce representative sections for comparison with radiographs taken during life, and also to demonstrate the distinctive

feature of focal emphysema. The method is applicable to other organs, particularly the liver, kidney and heart. The paper-mounted specimens can be stored in files.

The method consists of distending the lungs by running a solution of formalin and sodium acetate into the bronchi and when fixed the organs are cut into 1 to 3 cm. thick slices, which are washed and embedded in gelatin. The gelatin is hardened either by formalin or by freezing and sections 300 to 500μ are cut with a special microtome. The natural colours are preserved by the use of mono-phenyl ether of ethylene glycol.

The fixation of lungs in an expanded position and the examination of thin slices, whether cut free-hand or by a microtome has revealed pathological changes which might easily have been overlooked with the more usual method of a few cuts into the collapsed lung at the time of autopsy.

J. GOUGH.

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CHAPTER X

THE LIVER

It is a common belief that the multilobular portal cirrhosis of Laennec and acute yellow atrophy of the liver are due to the *direct action* of toxic substances on *healthy* liver cells, yet no toxic substance is known which will regularly produce in animals an exact copy of human portal cirrhosis, and the comparatively few poisons which occasionally produce acute yellow atrophy do so in a highly irregular and unpredictable fashion.

In 1932 experimental work provided the first hint that dietary deficiency may play a vital part in the production of these hepatic diseases. It became clear, for instance, that liver cells are unable to participate in the normal metabolism of fat unless the diet contains adequate quantities of *choline* or, in the place of this organic base, a sufficiency of the sulphur-containing amino-acid *methionine*, one of whose several functions is to facilitate the *in vivo* synthesis of choline. It was then discovered that long-continued deprivation of choline or of proteins containing methionine eventually results in progressive and uniform fibrosis of the liver very closely resembling that found in human portal cirrhosis.

From 1935 onwards experimental work provided more and more evidence that acute yellow atrophy may also be conditioned by dietary deficiency. By 1944 there was ample evidence that this disease could be regularly produced in animals by protein deficiency, and in 1945 Glynn, Himsworth and Neuberger demonstrated that the administration to rats of diets deficient only in the sulphur-containing amino-acid cystine resulted after a latent period in the abrupt development of a grave illness characterized by the sudden death of liver cells on a massive scale. This experimental acute massive necrosis of the liver in cystine-deficient animals is histologically indistinguishable from acute yellow atrophy in man.

The striking differences between the liver injury found in the choline-deficient animal on the one hand and in the cystine-deficient animal on the other will be stressed in this chapter. These differences are so striking that sudden death of liver cells may be assumed to play no part whatever in the production of

the fibrous liver of human portal cirrhosis, whilst human acute yellow atrophy and the scarring and hyperplasia which may follow it are the direct outcome of acute necrosis of liver cells on a massive scale and are unrelated to disordered fat metabolism. The irregularly scarred liver following acute massive necrosis is often described as cirrhotic. Very considerable confusion has resulted from this usage and it would be of material assistance to our better understanding of liver disease if the word " cirrhosis " were strictly limited to describe the liver in portal cirrhosis on the one hand and the large green finely granular liver of obstructive biliary cirrhosis on the other. The experimental proof that dietary deficiency plays so vital a part in liver disease suggests that the action of certain liver poisons may be conditioned by the state of health of the liver cells at the time of attack. This question will also be discussed.

Acute massive necrosis of the liver must be clearly distinguished from acute zonal necrosis, a common and usually mild lesion peculiar to the liver, and long familiar to pathologists. One variety of zonal necrosis is now recognized as being the essential lesion of acute infective hepatitis, which was responsible for a pandemic of febrile jaundice in the years 1942, '43 and '44. The many problems raised by the investigation of this disease will also be discussed.

These and other current problems in hepatic pathology have been reviewed at length by Himsworth (1947).

FATTY INFILTRATION AND PORTAL CIRRHOSIS

This new chapter in experimental pathology was opened in 1924 with the observation by physiologists that after pancreatectomy in dogs the liver became grossly infiltrated by fat. A similar but naturally occurring sequence had been previously observed in man (Hadfield and Clarke, 1924). In 1932 it was discovered by Best that the administration of *choline* restored the livers of these experimental animals to normal. It was then found that simple choline deficiency in normal animals rapidly produced such a gross degree of fatty infiltration of the liver that its function deteriorated. Choline and chemically related bases were then grouped together by Best and his co-workers (1933) as *lipotropes* or *lipotrophic substances* capable of preventing the deposition and excessive accumulation of neutral or cholesterol fats in the liver or of accelerating their disappearance if fatty infiltration were present.

Lipotrophic Substances. Prior to its utilization by the tissues neutral fat from the storage depots is carried to the liver where it is phosphorylated, one of its fatty-acid groupings being replaced by a phosphoric acid-choline complex to form a phosphatide which is then desaturated and its short fatty-acid chains subjected to β-oxidation. Within the limits of its functional capacity the liver promptly subjects to phosphorylation any neutral fat which may reach it. Of the total fat of the normal liver, amounting to about 4 per cent., only one quarter is neutral fat, presumably about to be phosphorylated, the remainder being phosphatide. *Phosphorylation is thus one of the basic functions of the liver cell and the choline molecule an integral factor in carrying it out.*

An excessive amount of neutral fat (glyceride) accumulates in the liver on a high fat or on a high carbohydrate diet producing the "fat-fatty liver" in which the glyceride content may be increased up to seventy times the normal. An excessive accumulation of cholesterol ester takes place on a high cholesterol diet ("cholesterol-fatty liver"). Fatty infiltration also takes place in the early stages of starvation, the total fat rising to 20 per cent., the increase being entirely due to neutral fat. A "fat-fatty liver" is common in experimental and clinical diabetes. In all these conditions the administration of choline in adequate quantities is followed by reduction or disappearance of the excess of fat or cholesterol ester. The amount of choline required to exert a full lipotrophic effect in gross fatty infiltration is small, amounting to 5 mgm. per diem in the rat. On the other hand, the administration of a choline-deficient diet is rapidly followed by gross fatty infiltration. Choline, by virtue of its protective lipotrophic effect on the metabolism of the liver cell, must therefore be regarded as an essential dietary constituent.

Lipotrophic Action of Proteins and Amino-acids. The next step in these investigations was the discovery by Best and Huntsman (1935) that casein has a lipotrophic action which could not be explained by the small amount of choline which it may possibly contain as a contaminant, and it was then shown for the first time by Channon and Wilkinson (1935) that a low protein diet alone can produce intense fatty infiltration of the liver, the excess of fat disappearing on the addition of an appropriate quantity of choline-free casein to the diet. Since then Channon and his co-workers (1938), investigating the lipotrophic effect of a number of choline-free proteins, found that egg albumin, beef muscle protein and edestin (from hemp seed) were nearly as active as casein but that fibrin and gliadin (from wheat gluten) were definitely less active. The lipotrophic effect of gelatin and zein (from maize) was inconspicuous.

The lipotrophic action of proteins is most readily explained as being due to their constituent amino-acids, and the above results can be correlated with a series of observations on the lipotrophic effect of individual amino-acids. The first to be investigated were the sulphur-containing amino-acids—cystine and methionine. The action of cystine was striking (Curtis and Newburgh, 1927; Beeston and Channon 1936). Far from preventing an excessive deposition of fat in the liver, cystine quite rapidly causes gross fatty infiltration. On the other hand, and in striking contrast with this discovery, methionine was

found to exert precisely the opposite effect (Tucker and Eckstein, 1937) having a protective lipotrophic action amounting to about one-twelfth that of choline. Amino-acids not possessing the sulphydryl grouping have shown no lipotrophic or anti-lipotrophic action. It seems, therefore, that the lipotrophic action of any protein after proteolysis and absorption as amino-acids is proportional to its content in lipotrophic methionine. Choline and methionine have obviously a profound influence on the metabolism of the liver cell and stand in the closest *in vivo* biochemical relationship with each other. Du Vigneaud and his co-workers (1939) and du Vigneaud (1941) point out that methionine has a labile methyl group, and they produce experimental evidence which suggests that this is probably responsible for the biosynthesis of choline by transmethylation. Using labelled elements, heavy hydrogen and radio-active sulphur, they showed that the labile methyl group of methionine is transferred intact to build up the choline molecule. This would explain the weaker and more delayed lipotrophic action of methionine compared with choline. It will be recalled that methionine is an essential amino-acid and that cystine is not. The relatively high lipotrophic action of milk casein is, in all probability, related to its high content of methionine, at least 90 per cent. of its sulphur being contained in this amino-acid.

Fatty Infiltration and Cirrhosis. The histological appearance of the parenchyma of a liver heavily overloaded with fat strongly suggests the existence of functional impairment with decreased capacity to consume oxygen and store glycogen. On general principles this should lead to disuse atrophy and fibrous replacement, and the first clear indication that diffuse fibrosis is the natural outcome of long-continued fatty infiltration came from Chaikoff and Connor (1938), who noticed that the fatty livers of depancreatized dogs maintained on insulin gradually lost their excess of fat and became increasingly fibrous. After periods varying from two and a half to five years the liver shows the typical changes of advanced portal cirrhosis. Similar results were then obtained by Chaikoff and Connor (1940) and by Chaikoff and his co-workers (1943) in *normal* animals maintained on high fat diets. The fibrosis originates in the portal tracts and is equally diffused throughout the liver. It starts insidiously as an increase in the fine interlobular argyrophil reticulin fibrils and slowly progresses until groups of lobules are enclosed in the meshes of a coarse fibrous tissue net. As the fibrosis advances the accumulated fat in the liver cells disappears. In the later stages single lobules become enclosed and groups of cells in the periphery of these lobules are cut off. In the fibrous tissue bands proliferation of the bile ducts is conspicuous. Eventually the liver becomes uniformly reduced in size, tawny yellow in colour, firm in con-

sistency, and its whole surface evenly studded with well-defined but equally-sized nodules. The morbid anatomical picture is a remarkably close copy of Laennec's multilobular portal cirrhosis so often associated in man with chronic alcoholism. It cannot be too strongly emphasized that during this process acute necrosis of the liver parenchyma is conspicuous by its absence. Himsworth and Glynn (1944b) lay particular emphasis on the slow progress of this variety of experimental dietary cirrhosis during which the animal shows a gradual and insidious deterioration of health over six to twelve months or more. There is no acute illness, and death usually takes place from intercurrent infection. They also stress the essential differences between this lesion, conditioned by long-continued and heavy fatty infiltration of the liver, and multiple nodular hyperplasia, which is always preceded by acute massive hepatic necrosis and is characterized by an irregularly scarred liver whose surface and section is studded with irregularly distributed coarse regeneration nodules varying very considerably in size and often in colour, some being tawny yellow, others green.

Human Malnutrition and Fatty Liver. For several years Gillman and his co-workers have been investigating the acute and chronic malnutrition of severe degree which is widely prevalent among the dark-skinned people of South Africa, many of whom are obliged to exist on a staple diet of maize meal supplemented occasionally and irregularly by beans, other vegetables, and fermented whole cow's milk. They stress the high incidence of hepatic cirrhosis and primary hepato-cellular carcinoma among young South Africans. In native populations suffering from severe malnutrition they have shown that extensive liver damage is invariably present even in children. The clinical course is characterized by attacks of a pellagra-like illness, and the liver eventually becomes cirrhotic (Gillman and Gillman, 1944, 1945). Severe hepatic changes have been produced in rats maintained on the same diet of maize meal and soured milk which produces human malnutrition in South Africans (Gilbert and Gillman, 1944 ; Gillman, 1944 ; Gillman *et al.*, 1945). The changes found in the liver in these animals were :

1. Diffuse enlargement with extensive accumulation of fat in 80 per cent.

2. Portal cirrhosis with variable degrees of fatty infiltration in 20 per cent., produced in approximately 150 days—corresponding to a period of four to five years in man.

3. Diffuse sinusoidal distension of the liver liable to give rise to

severe atrophic changes in the parenchyma and not infrequently resulting in lobar atrophy with or without lobar absorption.

The third change may well be a peculiarity of the rat liver and related to the striking anatomical separation of its four lobes, each of which carries its own separate venous and arterial blood supply. Fusion of the hepatic lobes in man possibly accounts for the rarity of any comparable lesion in human pathology.

Gillman and his co-workers lay particular emphasis on the conspicuous absence in their animals of acute diffuse necrosis and its sequels. The lesions produced are quite clearly comparable to those produced by deprivation of lipotrophic factors induced by methionine deficiency, the result of a low intake of first-class protein over a relatively long period.

Before and during the period of Gillman's investigations a number of other reports has appeared in the literature which make it abundantly clear that severe malnutrition with gross fatty infiltration of the liver is alarmingly common throughout the dark-skinned races of the whole of tropical Africa, where gross fatty infiltration of the liver is frequently found in both children and adults, in association with a series of clinical states all of which are clearly and directly attributable to dietary deficiency. Thus a grossly enlarged and intensely fatty liver with a striking absence of subcutaneous fat may be associated with nutritional diarrhœa, nutritional anæmia, many of the signs and symptoms of pellagra, or a combination of these conditions (Williams, 1933). Trowell (1937) and Trowell and Muwazi (1945), working in Uganda, call this association *malignant malnutrition*, to emphasize the fact that it is frequently incurable, is often found in infants and children in association with a series of definite signs and symptoms and that, if the child survives, the liver eventually becomes cirrhotic. There is no question of the wide incidence of malignant malnutrition in tropical Africa, where pathologists rarely see a completely normal liver in Africans at any age, and where the most common carcinoma is a malignant hepatoma.

All authors stress the serious prognosis of malignant malnutrition in the children of economically impoverished populations. Although more thoroughly investigated in Africa, it is likely that malignant malnutrition may well prove to have a world-wide distribution. Lack of subcutaneous fat, high-grade fatty infiltration of the liver, nutritional diarrhœa with œdema, and skin lesions resembling pellagra has a 90 per cent. mortality among children in Uganda, and there is no therapeutic response to any form of

vitamin therapy or to blood or serum transfusion. In 1944, Gillman and Gillman achieved a striking therapeutic success by the administration of ventriculin. In severe cases the liver becomes free of fat within two weeks and there is a dramatic and rapid reduction in the degree of œdema. This discovery must have a fundamental metabolic significance and it certainly seems justifiable to regard ventriculin as a powerful lipotrope.

Human " Alcoholic " Cirrhosis. There appears to be no reason why the experimental transition from fatty infiltration of the liver to diffuse fibrosis should not be applied to explain the pathogenesis of human portal cirrhosis associated with chronic alcoholism. There is quite clearly a resemblance between the insidious course of experimental portal cirrhosis produced by high fat or high carbohydrate diets, terminating in secondary infection and showing none of the manifestations of liver failure, and the similar slow deterioration of health of the human portal cirrhotic in whom the major manifestations of hepatic failure are inconspicuous and the presenting signs the result of portal obstruction. Fatty infiltration of considerable degree is in all probability the invariable forerunner of alcoholic portal cirrhosis. In a considerable percentage of cases of alcoholic cirrhosis dying before the liver has reached the shrunken end-stage of the disease the liver is still enlarged and contains a gross excess of fat easily visible to the naked eye (" fatty cirrhosis," see Fig. 10/1). The alcoholic burns alcohol in place of fat ; for economic and other reasons his diet is often deficient in the lipotrophic factors contained in first-class protein. On this basis, therefore, the old assumption that alcohol has a direct and specific injurious action on the liver cell becomes superfluous and alcoholic portal cirrhosis falls into line with alcoholic " pellagra " and " beri-beri " and with Wernicke's encephalopathy as a dietary deficiency conditioned by chronic alcoholism.

DIETARY DEFICIENCY AND HEPATIC NECROSIS

Experimental Dietary Necrosis. During recent years many papers have been published describing the production of severe hepatic disease in animals by the administration of abnormal diets. Although it is difficult to analyse some of the results obtained, it is quite clear that the successful experimental production of multilobular portal cirrhosis has been achieved by maintaining a state of gross fatty infiltration of the liver over a long period (Rich and Hamilton, 1940 ; Lillie *et al.*, 1941 ; Webster, 1941 and 1942 ; Blumberg and Grady, 1942 ; Chaikoff *et al.*, 1943). This work has already been discussed,

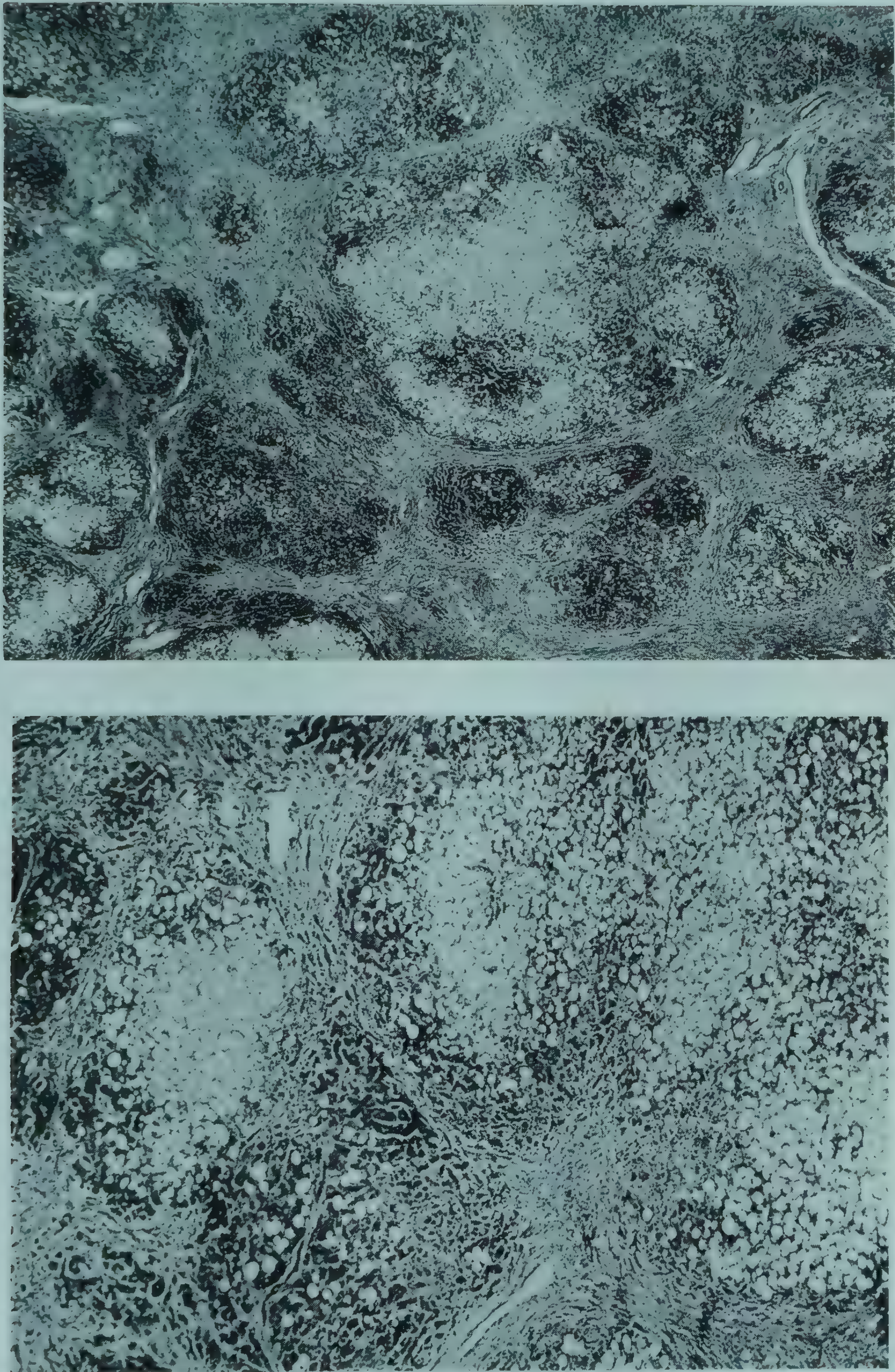

Fig. 10/1. Liver in Chronic Alcoholism. Showing a combination of gross fatty infiltration and portal cirrhosis.

[*To face page* 244.

but it is necessary to repeat that in several of these experiments portal cirrhosis together with varying degrees of residual fatty infiltration were the only lesions produced, and there is a singular lack of written or pictorial evidence that sudden death of liver cells—*necrosis*—was produced. It seems reasonable to conclude, therefore, that the fibrosis in portal cirrhosis is not the result of necrosis of liver cells but a consequence of slow *nutritional atrophy* of cells lying at the periphery of groups of lobules which, although held in the meshes of a wide-meshed fibrous net, show no very serious disorganization of lobular architecture. On the other hand, some workers describe the production of a variable but sometimes severe degree of necrosis and post-necrotic scarring (Gyorgy and Goldblatt, 1939 and 1942 ; Lillie *et al.*, 1942 ; György, 1944). The necrosis was recognized but the typically irregular scarring which follows massive necrosis was unfortunately described by György and Goldblatt as " advanced cirrhosis." They supposed this to be a late stage of portal cirrhosis of severe degree. Fatty infiltration, acute necrosis, portal cirrhosis and advanced cirrhosis, in this order, were then concluded to form a sequence of pathological changes resulting from lipotrope deficiency. As will be seen later, acute diffuse or massive hepatic necrosis and post-necrotic scarring form a well-defined sequence in which lipotrope deficiency probably plays no causative *rôle*, while fatty infiltration and portal cirrhosis form another and separate sequence apparently conditioned by lipotrophic deficiency. The word " cirrhosis " in pathological nomenclature has much to answer for, and it would prevent a great deal of confusion if its use were strictly limited to " portal cirrhosis," conditioned by fatty infiltration, and to " biliary cirrhosis," produced by long-standing biliary obstruction.

An examination of protocols of experiments described in the above papers, in which the production of hepatic necrosis by deficient diets is reported, shows that no account was taken of a severe degree of protein deficiency present in many of the diets used. In some experiments the drastic reduction of dietary casein carried out to reduce the lipotrope content of the diets also reduced the total protein to a dangerously low level. The animals were fed *ad libitum* on basic diets each of which contained a known percentage of carbohydrate, fat and protein. This method of feeding takes no account of the animal's appetite. For instance, if a group of animals is given *ad libitum* a protein-poor diet, those with large appetites may consume a fairly adequate protein ration whilst those with poor appetites may suffer from grave protein deficiency. As might have been expected, the results of these experiments were irregular and unpredictable, and the lesions produced formed a very variable mixture of fatty infiltration, portal cirrhosis, hepatic necrosis and post-necrotic scarring with multiple nodular hyperplasia. In the following paragraphs a series of feeding experiments carried out by Himsworth and Glynn (1944a) will be described in which the weight of each variety of food consumed per animal *per diem* was known, and consistent, predictable, and highly significant results were obtained.

Massive Necrosis and Protein Deficiency. Himsworth and Glynn (1944a) produced acute massive hepatic necrosis in rats

solely by the administration of abnormal diets. Their results showed that :

(*a*) The one essential feature of the diets which produces the lesion is that they are deficient in protein and that other dietary constituents have no more than a modifying influence on the results.
(*b*) There is close correlation between the protein intake and the incidence, severity, and speed of development of the lesions.
(*c*) The lesions produced are apparently morphologically identical with naturally occurring acute massive necrosis (" acute yellow atrophy ") in man. In both conditions there is widespread sudden death of liver cells : the blood-vessels, bile-ducts and connective tissues escape.
(*d*) The lesions produced are not conditioned by fatty infiltration of the liver, are often found in its absence and, unlike fatty infiltration, are not prevented by the administration of choline. Portal cirrhosis is not produced by protein deficiency.
(*e*) Complete protein deprivation, as in starvation, does not produce hepatic necrosis, as the animal then metabolizes its own muscle proteins and thereby protects its liver cells.

All the animals in these experiments received the same adequate daily allowance of vitamins A and D, thiamine, riboflavin, pyridoxine, and calcium pantothenate, and all were given an equal daily ration of the same salt mixture. As necrosis developed on some diets and not on others, neither vitamin nor mineral deficiency could have played a part in its causation or prevention. In some experiments the diet was composed predominantly of carbohydrate, in others predominantly of fat. Necrosis was produced by protein deficiency with equal facility in both groups and was frequently produced in animals in which the fat content of the liver was normal. Choline in doses of 4·mgm. per rat per day did not prevent or delay the production of necrosis. These results are in opposition to György's conclusion that the factors responsible for the production of fatty infiltration and portal cirrhosis are also responsible for acute necrosis and post-necrotic scarring.

The degree of protein deficiency necessary to produce necrosis showed considerable variation. In the first place, *total protein deprivation was not followed by acute necrosis,* presumably because the animal then metabolized its own muscle protein, and severe protein deprivation (e.g., a daily ration of 100 mgm. of casein per rat per day) was not followed by necrosis, probably for the same reason. On the other hand, when casein was used as the only dietary source of protein, a reduction of daily intake varying from 200 to 500 mgm. per animal per day was

regularly followed by massive necrosis. With this degree of dietary deficiency the animal's own muscle proteins were spared and the dietary protein alone metabolized, but, at this level, it was unable to protect the liver. When the daily intake of casein exceeded 500 mgm. per animal the liver was completely protected.

Yeast was then used as the only dietary source of protein and considerable quantitative differences in its action were discovered. A daily intake of 1,000 mgm. of yeast protein per rat had no protective action and 100 per cent. of the animals given 500 mgm. *per diem* developed necrosis. These results clearly suggested that the protective action of dietary protein on the liver cell is due, not to the intact protein molecule, but to some constituent part of it which is present in larger quantities in casein than in yeast. Now the amino-acid methionine has been shown by Miller and Whipple (1942) to protect liver cells against the toxic action of chloroform; it is richly contained in casein but present in much smaller quantities in yeast. An experiment was then carried out in which methionine equivalent to 8 per cent. of the dietary protein was added to a protein-deficient diet containing yeast as the only protein source. This diet in the absence of methionine produced acute necrosis in 100 per cent. of animals taking it. The presence of methionine afforded complete protection. This result at once suggested that the protective action of dietary protein may be due to its contained methionine. This assumption could not be accepted until feeding experiments were carried out in which the only source of nitrogen was amino-acid mixtures.

Massive Necrosis and Cystine Deficiency. Hock and Fink, working in Germany, published a paper in 1944 which strongly suggested that the protective action of dietary protein on the liver was due to its contained *cystine*. They fed animals on diets in which the nitrogen : sulphur ratio was varied, and found that the severity of the lesions increased as the amino-acid sulphur decreased and that the addition of cystine to diets, which in its absence produced extensive lesions, afforded complete protection. Glynn, Himsworth and Neuberger, unaware of the work being carried out in Germany, published in 1945 the results of careful experiments in which the *rôles* of cystine and methionine were more directly investigated and clearly defined. They used a basic diet, the nitrogen of which was supplied entirely by a mixture of amino-acids modelled on the amino-acid content of casein. From this diet the following experimental diets were constructed : sulphur-free, high methionine, high cystine, low methionine, cystine with low methionine. Massive hepatic necrosis developed when sulphur-containing amino-acids were absent or inadequate in quantity, appearing between the thirty-fourth and fifty-eighth day and unaccompanied by any excess of fat in the liver. Cystine or methionine in adequate amounts were equally protective and

this was shown to be due to the free conversion of methionine to cystine, the administration of methionine ensuring the supply of cystine to the liver cells. Methionine-deficient rats provided with cystine did not develop massive necrosis, but a specific methionine deficiency was produced, characterized by rapid and serious loss of appetite, wasting, anæmia, hypoproteinæmia and excretion into the urine of a substance related to or identical with homogentisic acid. On diets containing small quantities of methionine but no cystine these changes were absent but typical acute massive necrosis developed. We may therefore conclude that experimental massive necrosis of the liver is a deficiency disease due to lack of cystine. It has been shown (György, 1944; Schwarz, 1944) that the sensitivity of animals to massive necrosis induced by cystine deficiency is considerably increased by an inadequate supply of *tocopherol* in the dietary fat.

Liver Changes in Cystine-deficient Rats. The pathological lesions found by Himsworth and Glynn in livers of rats, first as the result of protein deficiency and later as the result of cystine deficiency, appear after the animals have been taking an effective diet for several weeks. During this latent period they appear to be quite healthy; at the end of it they are suddenly struck down by an acute illness, approximately twenty-four hours after the onset of which the liver is found to be swollen, friable, deep yellow in colour, and to show all the classical features of acute massive necrosis ("acute yellow atrophy") as seen in man.

The necrosis was quite clearly massive, involving the whole of many adjacent lobules. In other areas an appearance superficially resembling zonal necrosis was found but lacking the consistent, orderly and precise zonal distribution seen after poisoning by chemical hepatotoxins. In other areas, the size of which varied in inverse proportion to the degree of deficiency, there was a conspicuous absence of liver cell necrosis of any type. The livers of animals killed on the third to the fourteenth day after the sudden appearance of acute symptoms showed a gradual development of the typical morbid anatomical picture of human subacute massive necrosis ("red atrophy") as the disintegrating necrotic parenchyma gradually disappeared to be replaced by vascular granulation tissue rich in phagocytic histiocytes. Several weeks after the onset of symptoms the liver was found to be deeply scarred, its surface covered by nodules of regenerating liver, and the whole organ distorted into fantastic shapes. There was no essential difference between this stage and "multiple nodular hyperplasia" as seen in man.

As in naturally occurring acute yellow atrophy in man, regeneration of the hepatic parenchyma in the healing phase of

experimental massive necrosis is an imperfect and precarious process. In some areas the hyperplasia ceases at an early stage possibly because of an inadequate blood supply ; in others the regenerating tissue is broken into and invaded by extensions of the granulation tissue ; at a later stage it succumbs to ischæmia consequent upon devascularization of this granulation tissue. It is not surprising, therefore, that those animals which survive the acute attack never regain robust health, some of them dying with jaundice, ascites and subcutaneous œdema a few weeks after the onset of illness, others dying in a second acute recurrence. This clinical background of acute massive necrosis in the experimental animal bears a very striking resemblance to that of the naturally occurring disease in man and its grave sequels as described by Cullinan (1939).

Partial Massive Necrosis. A striking and significant feature of the hepatic lesions produced by Himsworth and Glynn was that they were more intense in the territory receiving blood from the left branch of the portal vein than in that supplied by the right branch. Furthermore, in those animals in which the protein deficiency was of mild or moderate severity the lesions were largely limited to those lobes of the liver supplied by the left branch of the portal vein.

It was long ago believed that the blood supply to the right and left sides of the liver was distinct, but the work of McIndoe and Counseller (1927) provided anatomical proof for this belief. The main portal vein was injected by a solution of celloidin in ether and the celloidin allowed to solidify. The liver tissue was then destroyed by corrosion in strong acid and a celloidin mould of the portal circulation obtained. These moulds often fell apart into two approximately equal halves and injection of the right and left branches of the vein by solutions of different colour showed that the branches of these two vessels were unconnected except by capillary sinusoids. A similar bilaterality was shown to exist in the case of the right and left branches of the hepatic artery and the hepatic duct. The line of cleavage does not correspond to the arbitrary line of descriptive anatomy. It passes through the middle of the gall bladder fossa to the junction of the hepatic veins with the inferior vena cava and bisects the Spigelian lobe. Division of the liver into two parts along this line showed the two halves to be of approximately equal weight, and the surface of sections to be singularly lacking in large veins, arteries or bile-ducts. This demonstration of anatomical bilaterality in the liver becomes of particular significance when correlated with the work of Copher and Dick (1928) who have shown that there are two distinct streams of blood in the portal vein which run side by side without mixing. Various branches of the vein were injected by *intra-vitam* dye in the living animal and corresponding areas in the exposed liver became coloured. Blood from the spleen

was invariably carried to the left lobe, that from the small intestine to the right lobe. When the exposed portal vein was transilluminated in a dark room, after injecting dye into the splenic vein, a clean-cut linear division was seen between the narrow splenic and the broad mesenteric streams throughout the short length of the vein. Glynn and Himsworth (1944) injected India ink into the spleen of normal anæsthetized rats. Within a few seconds of injection blackening occurred in exactly those parts of the liver particularly favoured by the necrotic process.

It is of particular significance that the marked liability of the left lobe of the liver to be severely affected by dietary massive necrosis in animals has also been described in fatal cases of naturally occurring acute massive necrosis in man (Stewart, 1917 ; Turnbull, 1917 ; Miller and Rutherford, 1923 ; Bergstrand, 1930). The explanation offered by Himsworth and Glynn for the asymmetry of the liver lesions in their animals is based on the double stream circulation in the portal vein described above. Blood from the spleen and large intestine going mainly to the left lobe and obviously poor in cystine and other protein breakdown products must have correspondingly low protective value.

THE ACTION OF HEPATO-TOXIC SUBSTANCES

Liver cells are peculiarly liable to sudden death or acute necrosis, and a very large number of chemical bacterial and metabolic poisons can kill them. There are two distinct varieties of acute hepatic necrosis. One of these, *zonal necrosis*, is an extremely common hepatic injury which can be easily and regularly produced in normal animals by a large number of chemical substances but does not appear to result from dietary deficiency. In the very large majority of instances, unless the dose of the poison is overwhelming, it is a mild and transient injury and heals rapidly. It occurs as the essential and, almost always, mild hepatic lesion of acute infective hepatitis. In experimental animals its severity can be greatly increased, but its pathological character is not radically altered, by the administration of an appropriate poison to protein-deficient animals. A very small minority of human patients suffering from acute infective hepatitis develop and die from acute massive necrosis of the liver. As this latter disease has been produced experimentally by deprivation of cystine, it is tempting to suppose that the development of acute massive necrosis in these cases is conditioned by protein deficiency. This has not been proved, but there is some evidence in support.

Acute massive necrosis is a rare and fatal disease whose experimental production by cystine deficiency has already been described.

It most commonly arises spontaneously or in association with pregnancy toxæmia but may follow poisoning by certain industrial chemicals or may arise during the therapeutic administration of certain organic chemical substances. Only a minority of those exposed to the action of these poisons develop massive necrosis. Those who do not develop massive necrosis suffer no hepatic injury. Experimental evidence makes it probable that the hepatotoxic action of the chemical substances which occasionally produce acute massive necrosis in man is strictly conditioned by pre-existing protein deficiency, although it is difficult to prove this in many human cases.

CLASSIFICATION OF HEPATO-TOXIC AGENTS

Group I: Producing Zonal Necrosis

Chemical

Industrial —Carbon Tetrachloride. Phosphorus.
Therapeutic—Chloroform. Tannic Acid.

Intoxications. Hyperemesis Gravidarum. Eclampsia. Proteus toxin.

Infections. Yellow Fever. Acute Infective Hepatitis.

The following therapeutic agents have also been accused of producing a hepatitis of this type. They are given by injection, and the effects they appear to produce on the liver are now usually considered to be due not to any toxic action they may themselves possess but to accidental contamination of the agent, the needle, or the syringe by the ultra-microscopic infective agent of acute infective hepatitis, or a closely allied virus infection:

Arseno-benzol drugs. Antisyphilitic drugs containing Bismuth. Gold preparations. Penicillin. Insulin. Liver extract. Homologous blood, plasma and serum. Reconstituted dried plasma. Convalescent mumps and measles serum. Yellow fever and Pappataci vaccines (both of which may contain human serum).

Group II: Producing Conditioned Massive Necrosis

Industrial Chemicals. Trinitrophenol. Dinitrophenol. Dinitrobenzol. Trinitrotoluol. Tetrachlorethane. Chlorinated Naphthalene.

Therapeutic Drugs. Cinchophen (Atophan : Phenylcinchoninic acid). Plasmoquin. Atebrin.

Toxic Grain. Selenium poisoning.

ZONAL NECROSIS

Under controlled experimental conditions the action on the liver of the *chemical* substances in Group I, conforms to the following common pattern :

(*a*) All the animals in a batch fall ill together a few hours after the administration of the chemical. This suggests that these agents act *directly* on *healthy* liver cells.

(*b*) The cells are killed outright. The necrosis is highly *selective*, connective tissue and blood-vessels escaping ; it is also strictly *zonal*, the killing being confined to cells occupying a definite anatomical situation in the lobule, the lesion being central, mid-zonal or peripheral.

(*c*) Zonal necrosis affects every lobule without exception throughout the whole organ.

(*d*) Increasing the dose increases the size of the lesion, but does not alter its character. With lethal doses the animal dies rapidly, the zonal lesion becomes almost total, but its zonal character remains, and every lobule is affected to an equal degree. For this reason alone it is extremely doubtful whether acute massive necrosis can be regarded as a further stage in the development of zonal necrosis.

(*e*) After single sub-lethal doses, recovery is complete ; the dead cells are removed in two to three days and all the lesions heal completely and without scarring in one or two weeks ; this is partly due to the preservation of the lobular connective tissue framework which prevents *architectural collapse*, partly to the preservation of the blood supply and partly to the zonal nature of the lesion which leaves a sufficiently large number of unaffected liver cells in *each* lobule to carry out effective *lobular* regeneration.

(*f*) If repeated doses are given at such short intervals that the zonal lesions have no time to heal, a fine and typically uniform fibrosis results. If, however, the doses are spaced to allow healing to take place between them, no permanent hepatic lesion follows.

This sequence of changes in zonal necrosis has been clearly described by Cameron and Karunaratne (1936) in the case of the centrilobular necrosis of experimental carbon tetrachloride poisoning.

All experimental evidence is opposed to a prevalent idea that massive necrosis is a severe or fulminating variety of zonal necrosis determined by a heavy dose of the agent or by an exceptional lack of resistance to it. This will be appreciated if the characteristics of massive necrosis are compared with those of zonal necrosis in the paragraph above:

Experimental massive necrosis arises after a latent period which is often measured in weeks.

Zonal necrosis is not found.

Between the necrotic areas groups of lobules are present, some of which are normal, others, although partially destroyed, do not show zonally distributed lesions.

If the protein content of deficient diets known to produce widespread massive necrosis be progressively increased, the lesions become more and more confined and eventually almost completely localized to the left lobe of the liver. Severe deprivation produces no alteration in the essential character of the lesion. Even in animals dying in the acute phase there is a latent period before symptoms appear.

Recovery, which is usually temporary, is followed by gross and irregular scarring and multiple nodular hyperplasia. The regenerated liver shows marked architectural abnormalities and regeneration is often functionally inadequate.

Varieties of Zonal Necrosis. As with carbon tetrachloride the sub-lethal lesions produced by chloroform and tannic acid (Cameron *et al.*, 1943) are centrilobular in position and transient in nature, healing rapidly without fibrosis. The centrilobular necrosis of hyperemesis gravidarum has the same characters. In eclampsia this lesion may be found together with peripheral necrosis associated with the familiar hæmorrhages into the portal tracts. Necrosis in phosphorus poisoning and after injection of *Proteus* toxin is peripheral or periportal. The zonal necrosis of yellow fever is almost unique (Klotz and Belt, 1930). It tends to occupy the mid-zonal position, a ring of necrotic cells being seen midway between the portal tract and the centrilobular vein of every lobule. In fatal cases it is widespread and the zonal distribution obscured. Healing produces no disorganization of the lobular architecture. The liver lesion in all cases of acute infective hepatitis is a perfect example of centrilobular necrosis, identical in its general features with that produced by all the agents in this group. Thanks to the biopsy studies of Roholm and Iversen (1939) and Dible, McMichael and Sherlock (1943) and to observations on the livers of patients suffering from this disease but dying from some unrelated cause (see Figs. 10/4 and 10/5), it is now possible to say that the lesion affects every lobule and, in the large majority of cases, heals with little or no fibrosis. An identical lesion is found in patients developing jaundice after the injection of arsenobenzol compounds (Dible, McMichael and Sherlock, 1943) and other therapeutic substances, including human blood and blood products. The evidence that these hepatic lesions are due to the accidental transmission of the infective agent of acute infective hepatitis will be discussed in another section of this chapter.

Nature of Zonal Necrosis. We have seen that zonal necrosis may be due to the action of a series of infective, biological and chemical agents which differ widely in their nature and chemical

constitution. In spite of this the lesion itself conforms in a remarkable manner to a common pattern, and its precise and orderly anatomical localization at once suggests that the necrotic cells lie in a definite vascular territory within the liver lobule. Himsworth and Glynn (1944b) have proposed that the action of agents which damage the liver in this way may do so, not by directly killing liver cells, but by causing acute cytoplasmic swelling. On this basis it is conceivable that swelling of the cells at the periphery of the lobule may be severe enough to restrict the supply of blood and protective cystine to the cells lying at its centre ; the necrosis would then be directly due to local cystine deficiency produced by partial centrilobular ischæmia. Furthermore, the injury produced would clearly be accentuated if the cystine content of the portal blood were low. Himsworth and Glynn (1944b) apply this hypothesis to explain the development of acute massive necrosis in well-nourished patients in whom there can be no question of long-continued cystine deficiency. The part played by the vascular factor in the production or accentuation of liver injury is clearly of basic importance. Its wide implications have been described by Himsworth (1947). Several investigations have been carried out which show that protein deficiency has a decided influence on the severity of zonal necrosis, those in which chloroform was used being of considerable interest.

It has been shown by Miller and Whipple (1940, 1942) that normal well-fed dogs were able to tolerate deep chloroform anæsthesia for an hour with little clinical evidence of liver injury. In dogs which had been fasted for three days, this period of anæsthesia produced well-marked centrilobular necrosis involving 50 to 70 per cent. of the cells of each lobule, and was occasionally fatal. Dogs given a low protein diet over long periods became very susceptible to chloroform necrosis, twenty minutes' anæsthesia being fatal in the large majority ; protection was afforded by a single large protein meal and equally effectively by methionine, even when given three to four hours after anæsthesia. Pregnant bitches starved for three days before labour were deeply anæsthetized for an hour during labour. The bitches died fifteen hours later, and their livers showed typical centrilobular necrosis. The pups survived and their livers showed no trace of injury.

Massive Necrosis complicating Zonal Necrosis in Man. The striking pathological differences between zonal necrosis and massive experimental dietary necrosis at once suggest that there may be no ætiological relationship between them in spite of their close but very occasional clinical association. On this supposition

acute massive necrosis in man may be regarded as a condition which complicates not more than approximately 1 in 1,000 cases of acute infective hepatitis, and it is tempting to suppose that this rare complication is conditioned by protein deficiency. There is no direct proof of this, but there is some suggestive collateral evidence in its favour.

The higher incidence of massive hepatic necrosis in women is very probably due to its association with the pregnant state and the proverbial liability of pregnant women to develop deficiency states by ruthless physiological diversion of maternal food to the fœtus. A relatively minor degree of deficiency will obviously be exaggerated by nausea and vomiting. Approximately 0·5 per cent. of pregnancies are complicated by hyperemesis and approximately 0·2 per cent. by eclampsia, in both of which conditions widespread zonal necrosis of varying degrees is probably always present. Acute massive necrosis, however, is a distinctly rare complication of hyperemesis and eclampsia, and it usually arises in the absence of any infection or exogenous chemical substance which injures the liver. These facts suggest the operation of an extraneous factor which, having regard to the heavy demands for protein in pregnancy, may well be a deficiency in protein intake. Finally there is a number of scattered references in clinical literature which strongly suggests that acute infective hepatitis in pregnant women is more frequently complicated by acute massive necrosis than in males and non-pregnant women (quoted by Himsworth and Glynn, 1944b).

Clinical and epidemiological studies in Central Europe between 1914 and 1920 showed a surprising increase in the incidence of acute massive necrosis (Seyfarth, 1921 ; Strumpell, 1921). This certainly corresponded with several widespread epidemics of acute infective hepatitis, but there were reasons for supposing that the essential causative factor was the economic malnutrition of the war and post-war periods. The mortality rates in two different populations from acute massive necrosis of the liver during epidemics of acute infective hepatitis show remarkable variations. In one of these, composed of well-fed physically fit American troops inoculated with yellow fever vaccine containing contaminated human serum, the mortality rate from massive necrosis was 0·17 per cent. The other population, composed of poorly-fed Brazilian peasants given the same vaccine and having a similar attack rate of hepatitis, had a mortality rate from acute massive necrosis of 2·5 per cent.

CONDITIONED HEPATIC NECROSIS

The industrial and therapeutic chemical substances in Group II (p. 251) give rise to a variety of liver damage which apparently has little in common with the zonal necrosis produced by the infective, toxic, and chemical agents in Group I.

As a group their action can be illustrated by considering the effects produced by *trinitrotoluol*. This substance, handled on an extensive scale by munition workers, produces toxic symptoms by absorption through the skin, giving rise to cyanosis, dermatitis and gastritis in a high percentage of workers. About 25 per cent. of all workers at risk develop one or more of these symptoms and a much larger percentage show some signs of poisoning. In sharp contrast, not more than 0·2 per cent. of those fully exposed develop " toxic jaundice " due to extensive and often fatal acute massive hepatic necrosis which, in those who survive the acute attack, is followed by multiple nodular hyperplasia. The lesion, which is morphologically identical with acute massive necrosis produced by dietary deficiency, has been described by Stewart (1917) and Turnbull (1917). This low incidence of hepatic necrosis amongst the large numbers of workers exposed to the same hazard is highly typical, as also is the length of exposure before necrosis develops. The majority of cases arise after about two months' exposure, very few after less than a month, an occasional case after six months, but fatal cases are unknown after twelve months. On the other hand, it is well recognized that acute necrosis may occur some weeks after all contact has ceased.

Himsworth and Glynn (1942), using rats, have shown that trinitrotoluol given by injection does not produce hepatic necrosis in well-fed animals. Lethal doses produce an acute illness, but the liver shows no evidence of necrosis. They were able to produce severe necrosis by maintaining their animals on a high fat diet which was low but not gravely deficient in protein. In these experiments there was a latent period of ten to thirty days before necrosis was produced. The severity of the lesion could not be correlated with the amount of trinitrotoluol given but was related to the degree of dietary deficiency and its duration before administering trinitrotoluol. It was characteristic of chronic poisoning produced by trinitrotoluol in animals receiving a high protein diet that hepatic necrosis was absent. There is some evidence that trinitrotoluol increases the basal metabolic rate, and therefore the protein requirements. Himsworth and Glynn suggest that an increased metabolic demand for protein may play a part in the hepatic necrosis which has occasionally been reported in thyrotoxicosis. Other evidence suggests that trinitrotoluol may combine with amino-acids *in vivo* and by combining with cystine may inactivate it. The available evidence supports the conclusion that the action of cinchophen and other members of Group II is in all essential respects similar to that of trinitrotoluol.

It is therefore a reasonable supposition, admittedly in need of further experimental support, that the action on the liver of sub-

stances in this group is conditioned by protein deficiency. It is very probable that selenium damages the liver in this way.

Selenium Poisoning. Chronic poisoning by selenium is characterized by diffuse hepatic necrosis, post-necrotic scarring, and multiple nodular hyperplasia which is more marked in the left than in the right lobe of the liver. There is good evidence for supposing that selenium produces its effects by replacing the sulphur in the cystine molecule and thereby inactivating it.

Soil derived from the cretaceous shale found in certain parts of the Great Plain States of the United States of America (Dakota, Nebraska, Wyoming, Kansas and Colorado) is comparatively rich in selenium. In these regions water-soluble selenates are absorbed by plants to such an extent that they become toxic to animals and cause considerable losses in livestock, a low rate of egg production in poultry, and occasional manifestations of selenium poisoning in the farming population. Wheat grown in ordinary soil has a selenium content up to 1·5 parts per million, whilst cereals grown in South Dakota contain up to 30 parts per million or even higher. Grasses containing 10 to 20 parts per million of selenium will produce typical symptoms of chronic selenium poisoning in cattle, the common pathological manifestation of which is post-necrotic scarring and multiple nodular hyperplasia of the liver, chiefly affecting the left lobe (Moxon and Rhian, 1943).

The experimental investigation of chronic selenium poisoning by feeding animals on cereals grown in seleniferous soil very soon showed extreme variations in individual and species susceptibility which eventually proved to be conditioned by protein deficiency (Smith, 1939 ; Smith and Stohlman, 1940). In one series of experiments toxic wheat containing ten parts per million of selenium was given to twenty-one animals as part of a low protein diet (casein 10 per cent.) Every animal developed advanced post-necrotic scarring and nodular hyperplasia of the liver which proved fatal in the majority. The same selenium-rich wheat was given to another series of animals receiving a liberal protein ration (30 per cent. casein) ; no significant changes were found in their livers. Smith and Stohlman concluded that the toxicity of selenium was determined, not by the level of selenium intake but by the ratio of protein to selenium in the diet. A considerable percentage of animals maintained in a state of chronic selenium poisoning over a long period developed multiple hepatomata and low-grade primary liver cell carcinomata (Nelson *et al.*, 1943).

Earlier investigation of the selenium problem had shown that the selenium in grain and grasses is an integral part of the plant protein. Having regard to the close chemical relationship between selenium and sulphur, it is a reasonable speculation that selenium replaces sulphur in the molecule of sulphur-containing amino-acids and that it may replace the sulphur in the cystine molecule and thereby inactivate it. On this supposition chronic selenium poisoning becomes a conditioned cystine deficiency. This speculation awaits further experimental and chemical proof ; it is supported by the fact that selenium can replace sulphur in amino-acids *in vitro* (Painter, 1941).

ACUTE INFECTIVE HEPATITIS

This disease, a common, highly infectious acute specific fever characterized by febrile jaundice, a relatively long incubation period, a low mortality rate and a very variable attack rate, is probably due to a filtrable virus which is spread by human fæces and introduced into the body *via* the gastro-intestinal tract. It has been repeatedly transmitted to human volunteers, in a few instances by serial passage, but in all probability has never been communicated to animals.

"Catarrhal Jaundice." Acute infective hepatitis is certainly the disease described by Virchow in 1865 as "katarrhalischen icterus" and considered by him to be due to acute duodenitis causing obstruction of the common bile-duct at the ampulla by mucous exudate. This explanation backed by the name of a great authority remained unquestioned for over fifty years, chiefly because the majority of cases make a complete recovery and the rare fatal case exhibits the typical post-mortem picture of acute yellow atrophy. During the 1914–18 war, Eppinger carried out post-mortem examinations on three typical cases of catarrhal jaundice who had died of trauma, and found a lesion in the liver which he regarded as "acute yellow atrophy in miniature." Up to 1939 only four further post-mortem examinations had been reported, and although a lesion similar to that described by Eppinger was found in each of them, "catarrhal jaundice" was still used and considered to be an adequate descriptive title for the disease. In 1939 Roholm and Iversen examined 38 cases of varying severity, obtaining biopsy specimens by aspiration of the liver in living patients in all stages of the disease. Widespread acute zonal necrosis of the liver was found in all of them. In 1943 Dible, McMichael and Sherlock repeated, confirmed and extended this work, and more recently Lucké (1944) has described the healing process following a typical attack of the disease in 14 cases dying from trauma or unrelated disease. It is now quite certain that acute destruction of liver cells is present in every case of acute infective hepatitis and that the primary lesion in the liver affords a completely adequate explanation of the jaundice and biliuria. Furthermore, repeated examinations of the duodenal contents have never shown any cytological evidence of duodenitis. It is also quite certain that acute infective hepatitis in epidemic form is the same disease which in sporadic cases is known as catarrhal jaundice They are clinically and pathologically identical, and many epidemics of hepatitis have been traced to sporadic cases. The term "catarrhal jaundice" will always carry the implication that the disease is due to extrahepatic biliary obstruction, and for this reason alone, quite apart from its obvious inaccuracy as a descriptive title, it should be abandoned.

Epidemiology. Acute infective hepatitis is not a new disease; there are many accounts of epidemics in the literature of the last hundred years. In times of peace, children of school age are by far the most susceptible members of the community; epidemics tend to remain localized and the attack rate is low. During wars it strongly

tends to become widely epidemic or pandemic, spreading rapidly among troops and adult civilians. It has its highest incidence in forward areas and in camps heavily infested with flies. Epidemics are clearly associated with insanitary conditions and coincident epidemic dysentery. The French refer to the disease as *jaunisse de champs*, the Germans as *Kriegs* or *Soldatenkrankheit Icterus*. There were 52,427 cases with 231 deaths in the four years of the American Civil War; 4,062 in the German army in the Franco-Prussian

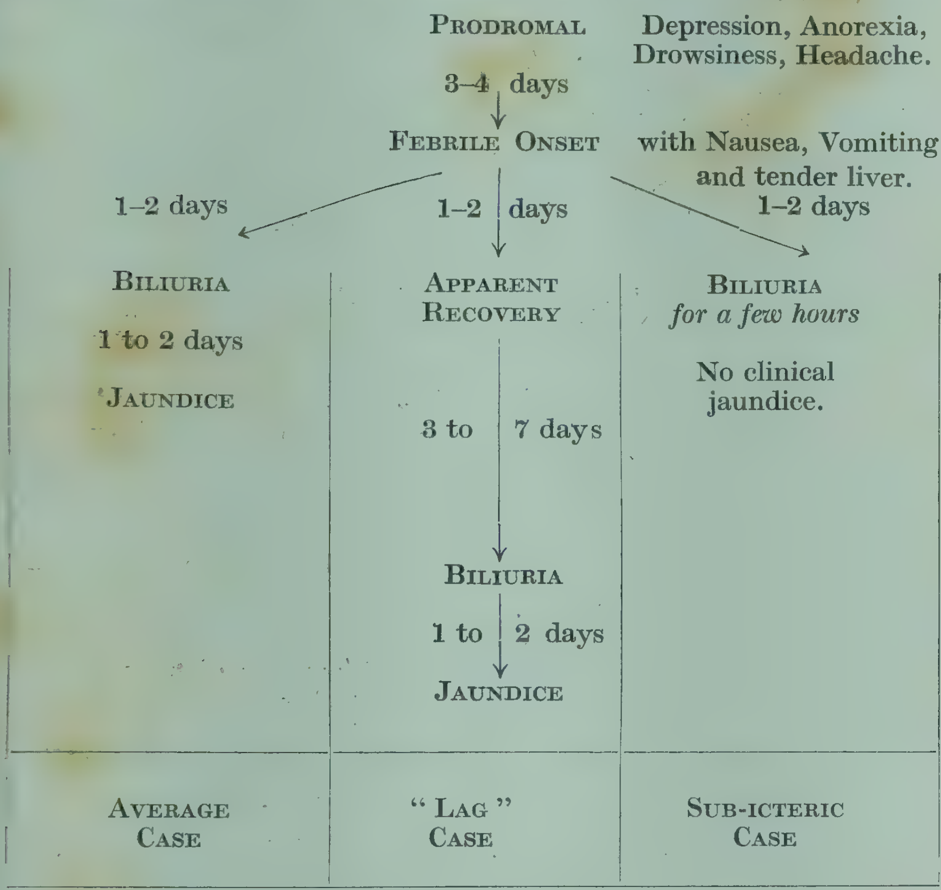

FIG. 10/2.

war; 5,648 in the South African war; large epidemics in Gallipoli and Mesopotamia in the 1914–18 war, and many civilian outbreaks in Europe and North America between 1921 and 1922, the most widespread being in the Scandinavian countries. It became pandemic in 1942, 1943 and 1944 in all theatres of war. The attack rate among troops living under bad sanitary conditions in the North African desert just before the battle of El Alamein reached 10 per cent. The disease spread extensively to civilian populations and became a major military and medical problem during these years.

The long incubation period and the absence of the leucocytosis are

suggestive of a virus origin. The period of infectivity extends from the indefinite non-specific onset to the development of jaundice; infectivity is rapidly lost after jaundice has appeared. An afebrile prodromal period of three to four days during which there is headache, anorexia, drowsiness and depression is followed by a febrile onset of one to two days' duration accompanied by gastro-intestinal symptoms (nausea, vomiting, more accentuated anorexia and tenderness of the liver). Bile, in quantities easily visible to the naked eye, then appears in the urine, to be followed in a day or two by clinical jaundice with some enlargement of the liver. The jaundice varies in degree from a hardly perceptible discoloration to a deep tawny orange. It disappears in less than a week in the majority of cases; sometimes it persists for three weeks, exceptionally for a month, and in rare and severe cases may last for as long as three months. In the average case the pre-icteric phase lasts for five to eight days (see Fig. 10/2). In a small but important group ("lag cases") this dangerous period of infectivity before jaundice appears is considerably prolonged. Such patients make an apparent recovery a day or two after the febrile onset, and the onset of jaundice is delayed for a further ambulatory period of three to seven days. In every epidemic there is a considerable

	Spirochætal	Acute Infective Hepatitis
Epidemiological background	A rat-infested environment.	Close contact, overcrowding or bad sanitation.
Percentage mortality	5 to 30.	0·1 to 0·2.
Incubation period in days	7 to 13.	20 to 40.
Blood examination:		
Leucocytes	Polymorphonuclear leucocytosis.	No numerical change or leucopenia.
Transmission	Leptospira in blood for first 7 to 10 days. Blood is infective to guinea-pigs, producing jaundice and "butterfly" hæmorrhages in lungs. (Urine also infective.)	Not transmissible to laboratory animals.
Antibodies	Specific agglutinins present.	Not demonstrable.

Fig. 10/3. Epidemic Febrile Jaundice.

but unknown proportion of cases which are ambulatory and whose prodromal and gastro-intestinal phases are mild and pass unnoticed. An unknown percentage of ambulatory cases never develop jaundice. In some of these, biliuria either does not appear, or the urine becomes dark for a few hours only and usually passes unnoticed by the patient. The lag cases and those which are ambulatory or ambulatory and sub-icteric make effective control of the disease by isolation extremely difficult and introduce a large element of fallacy into the estimation of the attack and mortality rates.

There are two other acute specific infections in which febrile jaundice is the essential feature of the clinical picture. Yellow fever is accompanied by a lesion in the liver, certainly due to a filtrable virus, and closely resembling that of acute infective hepatitis. Weil's disease (due to *Leptospira icterohæmorrhagiæ*) whilst less clearly related pathologically may however be readily confused with acute infective hepatitis, and it may be of value to compare their salient features (Fig. 10/3).

Transmission. Acute infective hepatitis has been transmitted on a number of occasions to human volunteers and successful passage has also been accomplished by the subcutaneous injection of bacteria-free blood and serum from cases of the disease in the pre-icteric phase (MacCallum and Bradley, 1944). By this route the incubation period tends to be remarkably long, varying from 60 to 100 days. Similar long incubation periods are also observed in the acute hepatitis which follows transfusion and intravenous therapy. Voegt, in 1942, claimed a successful transmission to a human volunteer by the oral administration of duodenal fluid from a case of acute infective hepatitis, and in 1944 McCallum and Bradley produced jaundice in three human volunteers by spraying a saline suspension of fæces previously extracted in alcohol on the nasopharyngeal mucosa. The incubation period approximated to that of the naturally occurring disease and varied from twenty-seven to thirty-one days. This experiment proved for the first time that the infective agent was present in the fæces. In 1945, Havens, Paul and Van Rooyen carried out successful transmission by feeding patients with fæces enclosed in gelatin-coated capsules, proving that the virus can enter the body via the gastro-intestinal mucous membrane. The incubation period in these experiments was twenty-eight days. Successful transmission has also been carried out by the administration of serum and of a Seitz-filtrate of the fæces given by the mouth.

Pathological Lesion. The primary hepatic lesion of acute infective hepatitis is constant and characteristic. It is the result of sudden death of a variable but often a considerable number of liver cells lying in the central part of every lobule throughout the

whole organ (see Figs. 10/4 to 10/6). This acute centrilobular necrosis, a variety of zonal necrosis, is highly selective, sparing the reticulin framework in which the liver cells lie as well as the hepatic connective tissue and blood-vessels and the bile-ducts (see Fig. 10/7). Although characteristic, the lesion is not specific, a wide variety of biological and chemical agents being capable of producing an identical variety of zonal necrosis. The severity of the lesion is often striking. In Dible's series it was estimated that in 12 out of 56 cases more than 50 per cent. of the liver cells were damaged. Spectacular as the lesion is, its relatively mild clinical background is, by contrast, surprising, and affords an excellent illustration of the magnitude of the functional reserve power of the liver. Invariably accompanying this primary lesion and persisting as long as the patient is jaundiced, there is conspicuous dilatation of the intracolumnar bile canaliculi, which contain " thrombi " of inspissated bile. The degree of bile retention is as variable as the degree of clinical jaundice.

In a few days after the onset of jaundice each minute focus of intralobular necrosis is fairly heavily invaded by mononuclear wandering cells and lymphocytes. Many of these cells migrate towards the portal tracts and the essential nature of the primary lesion is for a time obscured. Regeneration then commences and proceeds remarkably rapidly. Lucké has described this process in 12 cases dying from trauma or unrelated disease at varying periods after recovery from a typical attack of acute infective hepatitis, and in 2 similar cases in which biopsy specimens of the liver were obtained during laparotomy. The series included cases which had suffered and recovered from typical hepatitis of every degree of severity, the duration of the attack varying from fifteen days to six months, cases being fairly regularly spaced between these extremes. The interval between discharge from hospital and death from an unrelated cause showed a similar wide variation, from seven days to four months. In all cases the liver was macroscopically normal and showed complete preservation

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Fig. 10/4. Low power view of liver from a patient recovering from a relatively mild attack of Acute Infective Hepatitis. Death occurred nineteen days after onset of hepatitis from another unrelated disease. Pale linear areas are contracting foci of centrilobular necrosis.

Fig. 10/5. Hepatic lesion of Acute Infective Hepatitis. Case dying of another unrelated cause on the nineteenth day. High power view of preceding focus of centrilobular necrosis.

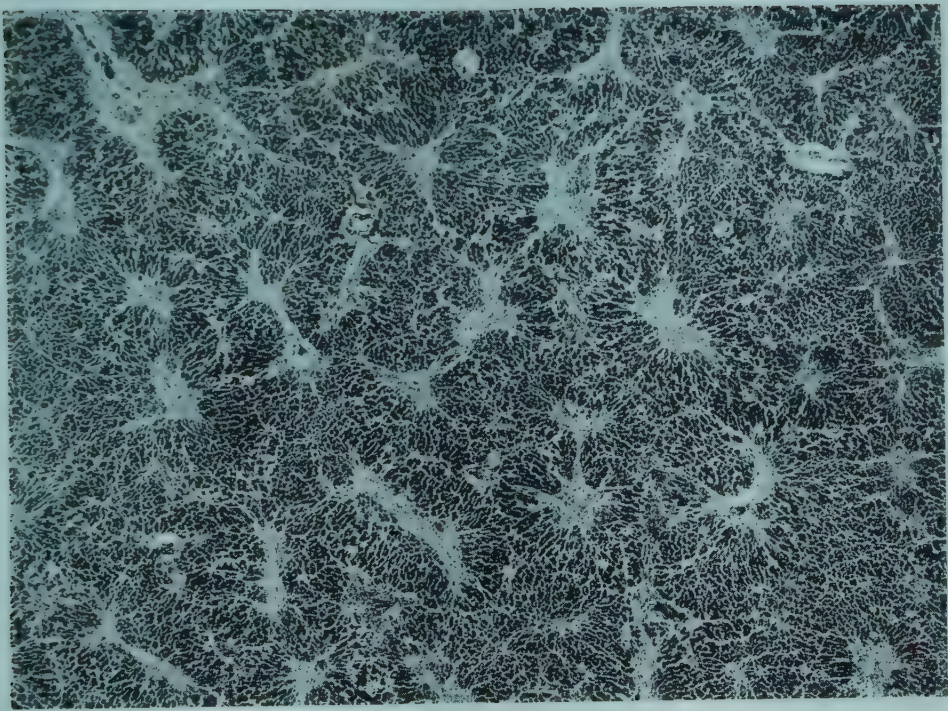

Fig. 10/4.

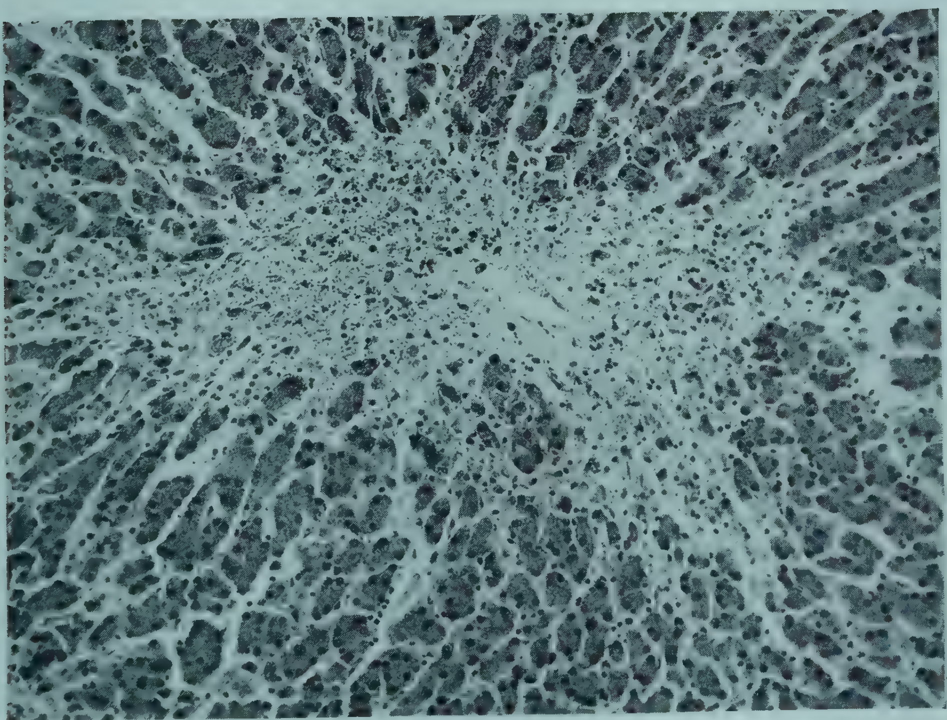

Fig. 10/5.

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FIG. 10/6. Hepatic lesion of Acute Infective Hepatitis. Mild attack; nineteenth day. Vessel in centre is centrilobular vein. The necrotic tissue around it is shrinking.

FIG. 10/7. Acute Infective Hepatitis. Liver lesion at the nineteenth day stained for Reticulin. Note that liver cells at the periphery of lobule are enclosed in a reticulin network and that although central cells have been killed their reticulin framework has survived.

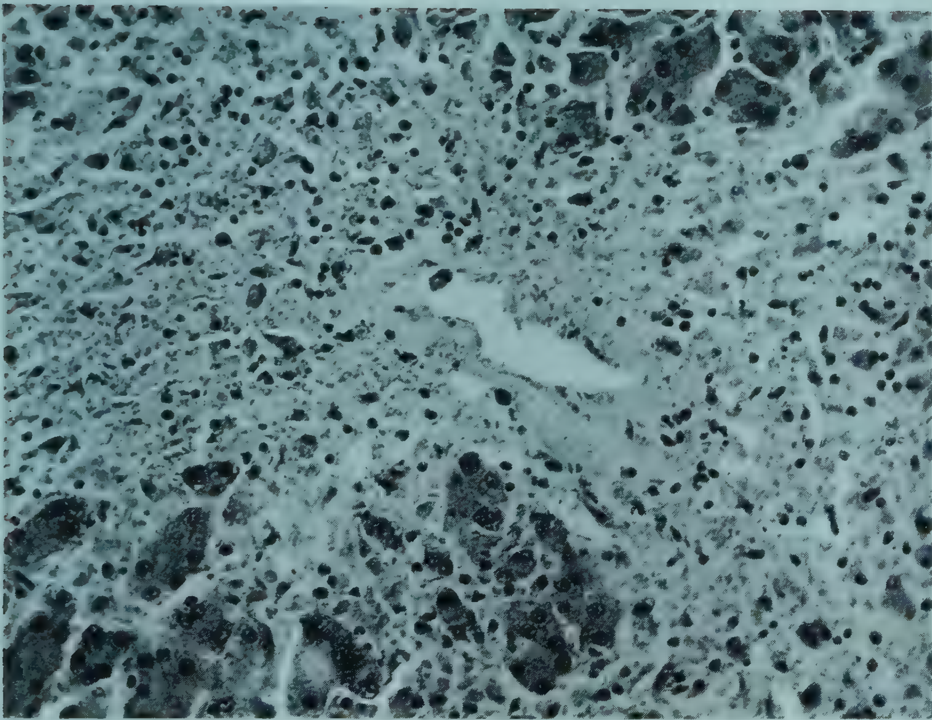

FIG. 10/6.

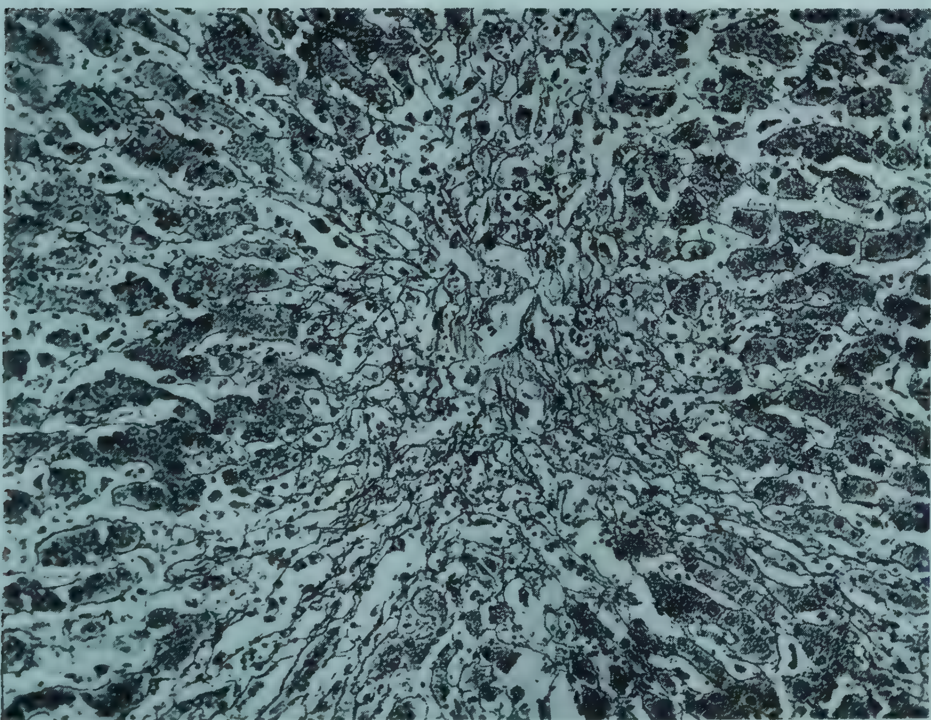

FIG. 10/7.

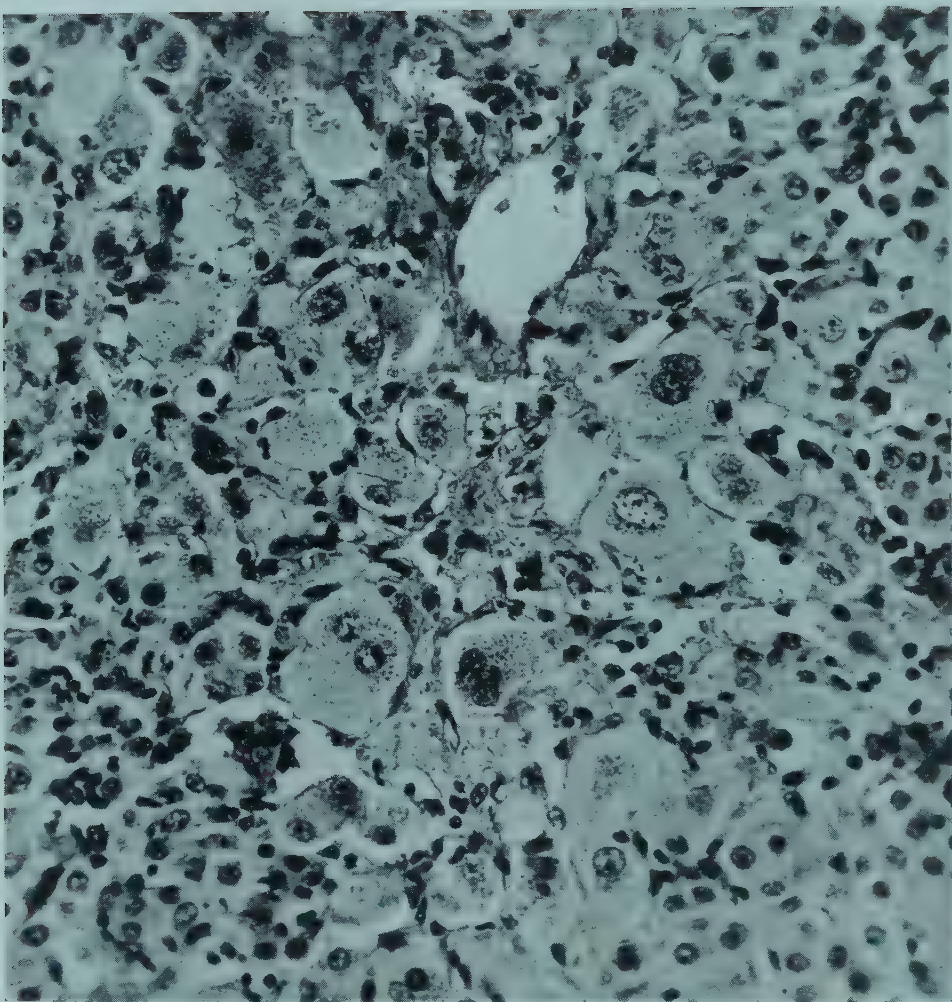

FIG. 10/8. Regeneration of central cells of a liver lobule thirty-eight days after an attack of Acute Infective Hepatitis of average severity. The centrilobular vein lies above and a little to the right of the centre. Around it are many large liver cells all showing a striking degree of nuclear activity.

of the architecture of the lobules and their reticulin framework. This was true even after recovery from jaundice which had lasted for six months. In cases dying within three weeks after clinical recovery regeneration of the liver cells in the central areas of the lobules had made considerable progress, but was still incomplete, and there was slight to moderate increase in cellularity of the portal tracts. Fibrosis at this stage was slight and focal, never diffuse. There was striking cytological evidence of liver cell regeneration in cases dying between fourteen and thirty days after clinical recovery. The central area of each lobule became packed with rapidly growing cells whose nuclei were active and hyperchromatic. Binucleate cells were frequent and multinucleate cells not uncommon. The high power view of these areas has a distinctly neoplastic appearance (see Fig. 10/8). As cells are regenerated at the edge of the necrotic centrilobular area they are pushed in long columns towards the centrilobular vein, which invariably remains unaffected, and the normal radial arrangement of the liver cell cords is quickly restored. In a week or two the regenerated cells show normal glycogen storage.

In all cases dying more than a month after clinical recovery regeneration was complete, but there was slight increased cellularity of the portal tracts. Two months after recovery there was some irregularity in size and shape of the lobules together with a moderate degree of swelling of the centrilobular cells, and an occasional binucleate cell still persisted. After this time it became more and more difficult to find any histological abnormality, and in none of the cases was there conspicuous scarring. It is safe to assume, therefore, that complete regeneration of the liver lobules occurs in the vast majority of all cases of acute infective hepatitis one month after clinical recovery. Similar structural repair and regeneration is found in approximately the same time in spontaneous and experimental yellow fever, in which there is necrosis of the mid-zonal cells.

Mortality. The mortality rate in cases exhibiting clinical jaundice varies from 0·13 to 0·44 per cent. (Lucké, 1944). If it were possible to include sub-icteric and mild ambulatory cases which occur in considerable but uncertain numbers in any epidemic this figure would probably be greatly reduced. It is not improbable that the true mortality rate is approximately 1 in 1,000 cases. Death is invariably due to hepatic failure, the result of acute massive necrosis of the liver (" acute yellow atrophy ").

There is no really significant histological difference between the

necrotic liver in fatal cases of acute infective hepatitis and that found in a very small proportion of cases of pregnancy toxæmia, in a few cases of poisoning by trinitrotoluol and cinchophen, and in the cystine-deficient rat.

Hepatic Failure. Lucké (1944) has published detailed accounts of the clinical histories and post-mortem examinations carried out in 125 fatal cases of acute yellow atrophy following acute infective hepatitis. The patients were adults, 78 per cent. being between twenty and thirty years of age. It is certainly a highly significant fact that there was no indication during the pre-icteric or icteric phases that the disease was destined to run any other than its usual benign course. In many instances the patient showed steady clinical improvement before the onset of fatal hepatic failure, was usually afebrile and not infrequently ambulatory. A fatal outcome was certainly not associated with a severe clinical attack of hepatitis.

The final phase was invariably unexpected, of sudden and dramatic onset and associated with grave nervous symptoms—lethargy, or coma alternating with restlessness and delirium. These symptoms were often accompanied or succeeded by persistent vomiting and, in 60 per cent. of cases, by rapidly developing ascites. The sudden development of grave nervous symptoms was always a clear clinical indication of the onset of hepatic failure. Death took place within twenty days of the onset of the final phase in 96 per cent. of cases ; within ten days in 60 per cent., and in four days or less in 35 per cent. Before death it was usually possible to demonstrate an appreciable reduction in the size of the liver. Hæmorrhages into the skin, hæmatemesis, epistaxis and bleeding from other mucous membranes were fairly common and probably due to a fall in prothrombin production by the damaged liver. The development of ascites was rather sudden and the volume of fluid tended to increase rapidly, reaching a volume of 2 or more litres. As might be expected from the gross degree of liver destruction which post-mortem examination reveals, there was often a significant fall in the protein content of the plasma. It was often found, however, that ascites appeared before any depletion of plasma protein was detected, and in some cases the plasma protein level remained unaltered. Furthermore, general œdema was a distinctly rare event. A fall in the colloid osmotic pressure of the plasma cannot, therefore, be the main cause of the ascites. Lucké suggests that acute disorganization, obstruction, congestion and probably stagnation of the portal

blood-flow through the liver due to rapid destruction and autolysis of its parenchyma may be the responsible factor. Endophlebitis of the efferent veins, which Lucké found to be a common accompaniment of acute yellow atrophy, would cause further embarrassment to the portal blood-flow. Lucké also points out that the parenchyma of the normal liver stores an appreciable volume of ingested water. This function must rapidly fail when the parenchyma is largely eliminated. In a considerable percentage of cases developing ascites there was gross and widespread œdema of the walls of the small and large bowel, most marked in the ileo-cæcal region.

The cerebral manifestations which herald the onset of fatal hepatic failure are difficult to explain. They resemble to some extent the symptoms of fatal hypoglycæmia which follow total hepatectomy in animals. It has been shown, however, that after large partial hepatectomies in animals death cannot always be attributed to hypoglycæmia, and the cerebral manifestations in fatal hepatitis in man seem to resemble more closely the condition produced by Baló and Korpássy (1932) by the administration of meat to dogs with an Eck fistula. Of eight such animals, six developed non-suppurative encephalitis which was ascribed to failure of detoxication by the liver of toxic products absorbed from the bowel. Lucké describes a similar but less intense variety of meningo-encephalitis with perivascular lymphocytic infiltration particularly affecting the brain stem which was present in 15 per cent. of his cases. The lesion found differs considerably from that affecting the brain stem produced by aneurin deficiency (Wernicke's encephalopathy). The balance of evidence is therefore in favour of regarding the nervous symptoms as being the result of a complex intoxication due to massive liver cell destruction rather than to deficiency of a single normal metabolic product.

The liver invariably showed a gross degree of acute massive necrosis, exhibiting in the more rapidly progressive cases the morbid anatomical picture usually described as idiopathic acute yellow atrophy, or, in cases dying after longer intervals, the changes of acute red atrophy with varying degrees of nodular hyperplasia. In four-fifths of the cases the liver was reduced in weight and bulk, usually weighing between 800 and 1,200 gm. (normal = 1,400 to 1,600 gm.). The maximum reduction in weight was found in rapidly fatal cases in which the liver was soft and flaccid, its surface finely wrinkled and its weight reduced to half the normal. The extraordinary rapidity with which the

parenchyma of the liver is killed, its necrotic remains liquefied by autolysis and the fluid products carried away is one of the outstanding features of the disease. Equally characteristic is the complete lack of uniformity and irregular distribution of the necrotic process. Large masses of the organ show total destruction of all liver cells, nothing remaining but the empty reticulin framework of the lobules. In other areas, the necrotic foci are small and between them lie groups of lobules well filled with viable parenchyma. The masses of liver which suffer total destruction undergo rapid shrinking, and it is significant that this is seen more often affecting the left lobe than the right.

In cases dying from the tenth day onwards there is clear cytological evidence of rapid regeneration of liver tissue from cells which have escaped destruction. In twenty days after the onset of liver failure, nodular masses of newly formed liver tissue of macroscopic size are clearly visible on the surface and in the substance of the incised organ. This tissue, which shows striking architectural imperfections, continues to grow until eventually large ivory-coloured or yellowish-green, firm, tumour-like nodules are formed. It is highly characteristic that these regeneration nodules show the widest variation in size, colour and consistency. They tend to be larger in the right lobe than the left. Their blood supply is precarious, and their biliary canaliculi and finer bile-ducts imperfect. Thus bile stasis is frequent in them and their cells often show slow degenerative changes due to relative ischæmia. The violent acute necrotic process which attacks normal cells of the liver lobules at the onset of acute yellow atrophy is rarely if ever found affecting the cells of the regeneration nodules. This may mean that acute massive necrosis is not, generally speaking, a recurrent process and, when liver failure develops after nodular hyperplasia is established, this is due to functional failure of the newly regenerated tissue possibly precipitated by devascularization of the granulation tissue in which it lies.

Lucké found in the kidneys in his series of cases the lesion described as " cholæmic nephrosis." In this condition, frequently found in diseases characterized by jaundice and liver failure, the kidneys are swollen and the epithelium of the convoluted tubules shows a variable, but often considerable, degree of necrosis which is rapidly followed by regeneration. Many chemically unrelated toxic substances, including perchloride of mercury, produce this change, and it is a reasonable supposition that the necrotic process

is due to toxic substances absorbed from the bowel which the severely damaged liver has failed to destroy.

HOMOLOGOUS SERUM HEPATITIS

In spite of the failure to transmit acute infective hepatitis to laboratory animals and the lack of any specific antibody-antigen reaction, there is now a large body of sound evidence that this disease, or a very closely allied infection, may be transmitted by any therapeutic measure which involves the administration of bacteriologically sterile blood, plasma or serum, from one human being into another by the intradermal, subcutaneous, intramuscular or intravenous routes. The donor usually shows no sign of ill health and apparently carries the causative icterogenic agent in his blood. The minimal infecting dose of blood or serum may be small—certainly of the order of 0·1 c.c. and probably considerably less. The disease may follow the administration of serum which has been repeatedly passed through a Seitz filter, stored in a frozen or dried state for many months (in one instance for four months at — 20° C.), preserved for several months in 0·2 per cent. tricresol or in 0·5 per cent. of a mixture of equal parts of phenol and ether. Heating the serum at 56° C. for one hour does not destroy the infective agent. The evidence is equally convincing that acute infective hepatitis may be transmitted by the minute trace of blood left in the barrel of a syringe when several patients are injected, even when the syringe is repeatedly rinsed in saline between injections, and that the febrile jaundice which may follow the intravenous injection of arsphenamine is due to the accidental transmission of a virus causing acute hepatitis.

The first observation pointing to this possibility was provided by an outbreak of jaundice in a Bremen shipbuilding yard in which out of a total of 1,289 persons vaccinated against smallpox by an arm to arm technique, 191 developed jaundice after an interval of several weeks, whilst no jaundice occurred in 587 others in the same shipyard who were vaccinated by calf lymph (Lurman, 1885 ; Hirsch, 1886).

Jaundice following Blood Transfusion. In 1944 MacCallum and Bauer inoculated eleven human volunteers subcutaneously with samples taken from 65 litres of pooled human serum from a blood bank. Four of the volunteers became jaundiced and in two, although there was no clinical jaundice, a sustained rise in the level of serum bilirubin was observed together with symptoms arising after the same incubation period as the cases which became jaundiced. Five more volunteers received serum by subcutaneous injection from a case of homologous serum jaundice ; two became jaundiced. Five others were inoculated intranasally with the same serum, one became jaundiced and one

developed a sustained rise in serum bilirubin. A considerable number of cases is now reported in the literature of jaundice following blood transfusion for shock, hæmorrhage, or peripheral vascular disease (Beeson, 1943; Morgan and Williamson, 1943; Steiner, 1944; Bradley *et al.*, 1944), and there is little doubt that a large number of unreported cases must have occurred. Having regard to the long latent period between transfusion and the onset of jaundice and the rapid evacuation and wide scattering of casualties, many cases must have been missed. The latent period varies between 49 and 114 days, the disease is usually mild, but an occasional case of fatal acute massive necrosis of the liver has been reported. Dible, McMichael and Sherlock (1943) in a study of biopsy specimens from two cases of transfusion jaundice could find no histological difference between the hepatic lesion and that found in acute infective hepatitis. It is probable that a virus resembling that of human acute infective hepatitis may be transmitted to horses during the administration of antitoxic sera. Stagsvold (1938) reported that 4 per cent. of a series of horses given anti-anthrax serum prepared in horses died of acute or subacute hepatic necrosis.

Hepatitis following Vaccination against Yellow Fever. During the second World War all British and American troops proceeding to areas where yellow fever is endemic were given a vaccine containing attenuated yellow fever virus suspended in human serum. According to published data 49,537 cases of acute hepatitis followed the use of this vaccine, the jaundice usually being noticed after reaching the endemic area. In the preparation of the vaccine, the virus is grown in a medium containing Tyrode solution and minced chick embryo. After repeated subculture this culture virus is grown in the living chick embryo, the affected embryo is minced, suspended in pooled and apparently normal human serum, filtered, adjusted to contain a standard amount of virus, frozen and dried. The jaundice is certainly not due to propagation of yellow fever, the clinical signs of which are lacking, fever often being absent, kidney involvement unknown, and the incubation period being quite inconsistent with yellow fever. The yellow fever virus cannot be demonstrated in the blood of early cases of vaccine jaundice, and the blood contains antibodies at an early stage of the illness. If yellow fever were the cause the disease would be widespread and the virus present in a virulent form in the large majority of the batches of vaccine issued. Investigation showed that only two out of 187 batches of vaccine were icterogenic on one occasion and eight out of eighty on another. The potency of the infective agent may be judged from the fact that each man received not more than 0·1 c.c. of serum by injection. A long latent period was observed between inoculation and the appearance of the jaundice or bile in the urine, the limits being three and a half to thirty-four weeks, the largest number occurring between six weeks and six months. The disease was rarely fatal, mortality varying from 1·45 to 2 per 1,000 cases, except in a series of cases in which the mortality in Brazilian peasants in one large series was 24·8 per 1,000. The possibility that dietary deficiency may have played a part in causing this high mortality cannot be overlooked. In fatal cases the liver showed acute massive necrosis, post-necrotic scarring or multiple nodular hyperplasia.

The disease has been transmitted to human volunteers by Oliphant, Gilliam and Larson (1943). Of 50 volunteers inoculated with an icterogenic strain of vaccine, 12 became jaundiced. Of 10 others inoculated with the same vaccine heated to 56° C. for thirty minutes, 2 became jaundiced, but after exposing the vaccine to ultra-violet light for two and a half hours not 1 of 10 volunteers became jaundiced. Of 30 volunteers inoculated subcutaneously with the pooled serum of 18 human cases of yellow fever vaccine jaundice, 8 became jaundiced, and of 14 injected with the pooled serum of cases of vaccine jaundice in the pre-icteric stage, 4 became jaundiced. It is a remarkable fact that so far no contact infections have been observed amongst the large numbers of people exposed to the 28,585 cases of vaccine jaundice in the American Army, in spite of the close contact of active service conditions.

The addition of human serum to yellow fever vaccine for use in the American Army was discontinued after the outbreak of the last large epidemic. Since this precaution was taken no jaundice has been notified (Badger, 1944).

" ARSENO-THERAPY " HEPATITIS

(" Syringe jaundice ")

Two distinct and apparently unrelated varieties of jaundice may occur during the treatment of syphilis by injection of organic arsenical compounds. The first and less common variety (Milan's syndrome : Erythema of the ninth day), arises constantly between the sixth and the fifteenth day after the first injection, is part of a general toxic response to arsenic and is considered to be a Herxheimer reaction. The second appears after a long interval varying between twelve and seventeen weeks after starting treatment. Its clinical characters closely resemble those of acute infective hepatitis. Its intensity varies from a mild illness in the large majority to rapidly fatal acute diffuse necrosis in a small minority. It is most improbable that this variety is due either to the action of arsenic on the liver or to syphilitic hepatitis. The variation in incidence of 2 to 46 per cent. in the same V.D. clinic from year to year is almost sufficient in itself to exonerate arsenic as the cause and there is no correlation between incidence and dosage, total amount of arsenic given, type of arsenical used, any particular batch of the drug or the method of manufacture. Arsphenamine therapy may be continued in syphilitics suffering from this type of jaundice without any ill effects. A syphilitic origin for the jaundice is equally unlikely. The maximum incidence of jaundice in untreated syphilis is less than 0·4 per cent., with no appreciable variation from time to time. Ruge (1925) produced strong epidemiological evidence that in the German navy the incidence of jaundice in syphilitics bore a definite relation to the incidence of infective (non-spirochætal) jaundice in the general population, and the rise in incidence among syphilitics in this country has been observed over a period when infective hepatitis was becoming increasingly common in the general population (Beattie and Marshall, 1944). Roholm and Iversen (1939) showed by biopsy that in arsphenamine jaundice there is centrilobular necrosis of the liver which differs in no essential from the hepatic change found in acute infective hepa-

titis. This has been confirmed by Dible and McMichael (1943) and by Dible, McMichael and Sherlock (1943) who were unable to correlate the histological changes with those produced by arsenic or syphilis.

It seems improbable that syphilis or arsenic predisposes the liver to attack by a specific virus. The incidence of jaundice following serum injections in large groups of patients whose livers were previously normal has reached 47 per cent. in the case of mumps convalescent serum, and 57 per cent. in the case of dried plasma for blood transfusion. The routine injection technique used in V.D. clinics before the possibility of transmitting acute infective hepatitis was fully realized could not be relied upon to prevent the transference of an infective agent (Bigger, 1943), and it has been shown that the incidence of jaundice in V.D. clinics can be substantially reduced by the introduction of aseptic technique (Salaman, 1944). Finally, MacCallum and Bauer (1944) transmitted the disease to human volunteers by inoculation of the serum from 2 cases developing jaundice at about the fourteenth week of arsenical treatment for syphilis, and at a time when the general incidence of infective hepatitis was at a low level. The incubation period of the transmitted disease varied from forty-two to eighty days. Attempts to transmit the disease by the administration of fæcal suspensions by the mouth were unsuccessful.

The Nature of Homologous Serum Hepatitis. It is still uncertain whether this condition is due to a virus identical with that producing acute infective hepatitis or to a different but closely allied infective agent. The problem has been investigated by Neefe and his co-workers (1945a and b) and by Havens (1946).

The disease is transmissible to man by subcutaneous injection of serum from human cases in the pre-icteric stage and has been serially passaged. The virus has been shown to be present in the circulating blood of an apparently normal human volunteer sixty days before the onset of jaundice and thirty-four days after inoculation with icterogenic human serum. Repeated experiments have shown that the virus is not present in the fæces, and administration of infective material by the mouth has never caused jaundice. The incubation period of the disease, whether accidentally or deliberately transmitted, is always long and of the order of 100 days or more.

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CHAPTER XI

RENAL FUNCTION

There have been notable advances in our knowledge of normal renal function during the last thirty years, and the application of this knowledge to the study of the kidney in disease has revealed unsuspected disorders of renal function, many of which are unaccompanied by any significant alteration of structure. It will be of interest, therefore, to preface our account of some recently-acquired knowledge of renal pathology, by a brief summary of the basic principles underlying the investigation of renal function in order to obtain an overall picture of the kidney as a living, working organ.

Physiological Considerations

The kidney's function is to maintain a normal internal environment. Waste products of metabolism and substances absorbed in excess of the body's needs are excreted by complex processes varying from substance to substance. In general, however, these processes can be summarized as follows : All substances in the plasma of molecular weight of less than approximately 60,000 are filtered at the glomerulus and pass down the tubular lumen. Some substances such as glucose, which are required by the body, are re-absorbed by the tubules and passed back to the blood. When the plasma glucose concentration is normal, the re-absorption is complete so that none of the substance is actually excreted (see Fig. 11/1). Other substances pass on down the tubule without any re-absorption and are excreted, e.g., inulin (see Fig. 11/2). Substances of a third group are filtered in the ordinary way and partly re-absorbed, e.g., water, sodium, urea, etc. (see Fig. 11/3). The proportion of each substance recovered from the glomerular filtrate is nearly always regulated by the body's needs. Other substances, constituting a fourth group, are actively excreted by the renal tubules as well as by a process of glomerular filtration. In the case of some of these, this tubular excretory process is so active that at low plasma concentrations the blood is almost completely cleared of the substance concerned, e.g., para-amino-hippurate, diodrast and penicillin (Fig. 11/4). Sub-

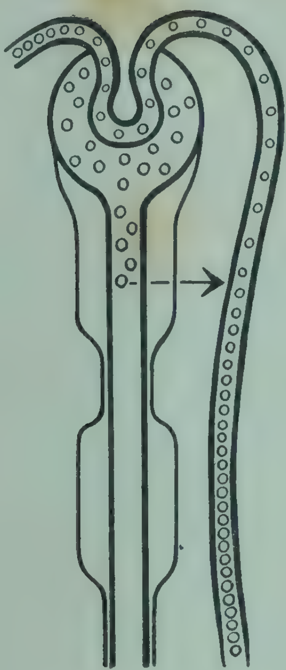

FIG. 11/1. Glucose.

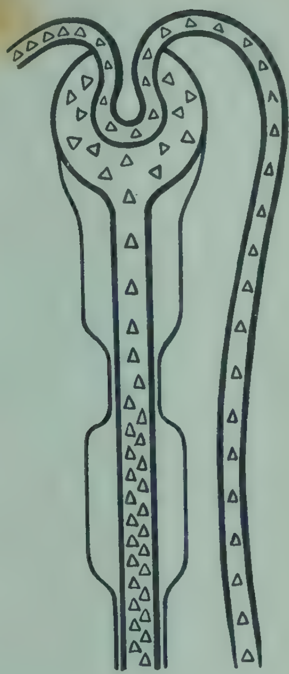

FIG. 11/2. Inulin.

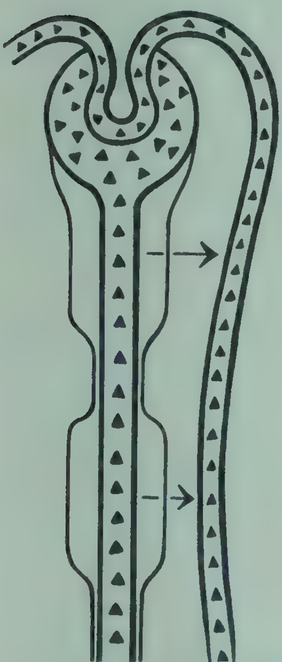

FIG. 11/3. Urea, Na, H_2O.

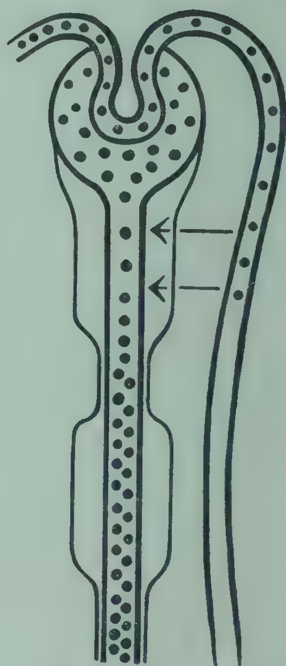

FIG. 11/4. PAH low blood level.

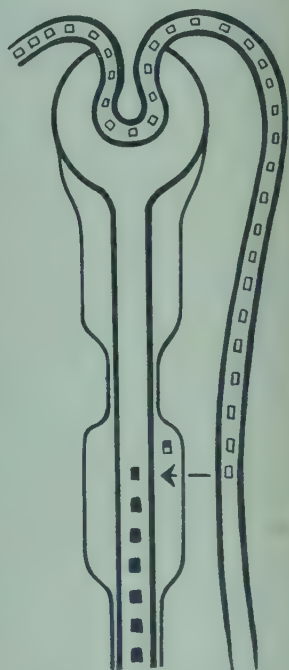

FIG. 11/5. NH_3 and H.

stances of a fifth group, exemplified by hydrogen and ammonia ions, are not present as such in the blood, but are produced from precursors in the plasma and excreted by tubule cells (Fig. 11/5). The secretion of hydrochloric acid by the stomach is an example of a similar process. In the case of ammonia, the precursor is an amino acid. The processes of glomerular filtration, tubular re-absorption and excretion, and in addition the rate of blood flow through the kidney can be quantitatively measured in man without operative interference. The principles of the methods of determining these functions and of certain other tests of renal function will be described.

THE MEASUREMENT OF DISCRETE RENAL FUNCTIONS

Glomerular Filtration Rate. Substances of molecular weight of greater than approximately 60,000 do not normally cross the glomerular membrane. As the molecular weight decreases these substances are filtered more and more freely until at a molecular weight of approximately 30,000 and below they pass the membrane so freely that their concentration in the glomerular filtrate is the same as in plasma. It is now known that a number of substances which are freely filtered in this way undergo neither re-absorption nor excretion by the tubule ; the best-known example of this type of substance is inulin, a polysaccharide having a molecular weight of approximately 5,000. The quantity of inulin or a similar substance entering the bladder per minute is, therefore, equal to the quantity being filtered per minute. The quantity entering the bladder per minute can easily be obtained by estimating its concentration (U) in bladder urine and multiplying this figure by the volume of urine excreted per minute (V). UV thus represents the amount of the substance entering the glomerular capsules per minute. This may be, for example, 10 mg. per minute. The concentration of inulin in glomerular fluid is the same as in plasma, so the amount of water accompanying this quantity of the substance can be determined by estimating the plasma concentration (P). In the example given, the plasma concentration might be 10 mg. per 100 ml. ; therefore, 100 ml. of glomerular filtrate contain the amount of the substance filtered, i.e., the glomerular filtration rate is 100 ml. per minute. Alternatively, 100 ml. of plasma have been cleared of the substance per minute. The simple formula $\frac{UV}{P}$ expresses

the above and will be recognized as the familiar clearance formula.

The substances which are excreted in this way, and whose clearances can, therefore, be used to determine glomerular filtration rate, include inulin, mannitol and thiosulphate in man and creatinine in the dog and certain other animals. The normal glomerular filtration rate in man (GFR) is 131 $\pm$ 21·5 ml. per minute. Approximate estimates of glomerular filtration rate can be obtained by determining the clearances of certain other substances. Creatinine is excreted in man mainly by a process of glomerular filtration, but in addition a small fraction of the creatinine appearing in urine is secreted by the tubules. This tubular fraction is so small in the case of endogenously produced creatinine that the endogenous creatinine clearance approximates very closely to glomerular filtration rate.

Urea is excreted by a process of glomerular filtration and a proportion of the filtered urea is re-absorbed. This re-absorbed fraction varies with the rate of urine flow, but at rates of urine flow of more than 2 ml. per minute it becomes fairly constant, so that the urea clearance is approximately seven-twelfths of the glomerular filtration rate. Determining the urea clearance will therefore give an approximate estimate of the rate of glomerular filtration. As urea is excreted purely by filtration, the blood level of urea tends to rise when the glomerular filtration rate is decreased. The height of the blood urea is therefore an indication of the level of glomerular filtration. The actual blood urea concentration is, of course, also affected by alterations in the rate of urea formation in the body, so that it is only a very rough indicator of glomerular filtration, but it is adequate for most clinical purposes.

Renal Blood Flow[1]. The amount of any substance entering the kidney through the renal artery per minute equals the amount leaving the kidney in the urine plus the amount returning to the body through the renal vein.

Let F = the volume of blood entering the renal artery in ml./min.
V = the volume of urine leaving the kidney per minute, ml./min.
A = the concentration of the substance in the arterial blood, mg./100 ml.

[1] I am indebted to Dr. K. G. Lowe for this explanation of the principle of renal blood flow determination.

R = the concentration of the substance in renal vein blood, mg./100 ml.

U = the concentration of the substance in urine, mg./100 ml.

Then the amount of the substance entering the kidney per minute is AF. The amount leaving in urine is UV and the amount returning to the body in the renal vein is R(F − V). In this expression F − V is the volume of blood flowing back through the renal vein which equals F the volume of blood entering the kidney by the artery *minus* the volume of urine (V) (see Fig. 11/6).

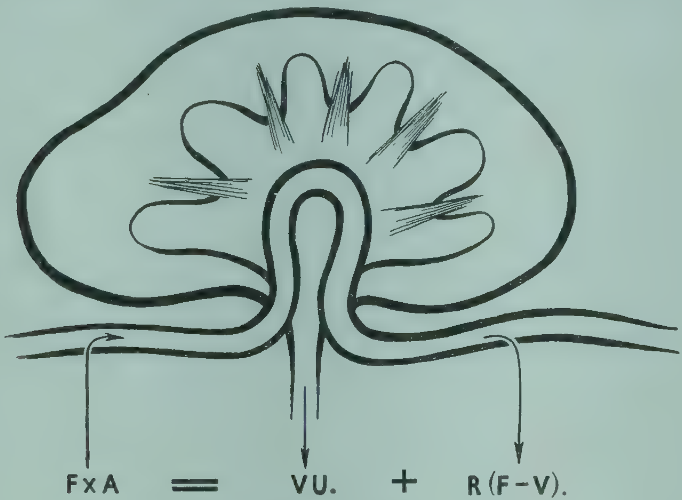

Fig. 11/6.

Thus : $$AF = UV + R(F - V).$$

This can be simplified to derive F, the arterial inflow—

$$F = \frac{UV}{A - R} - \frac{RV}{A - R}.$$

Therefore, by determining A, R, U and V, we can measure the renal blood-flow, provided the chemical estimation of any excreted substance is sufficiently accurate.

Fortunately, some substances when present in the blood in low concentrations are almost completely removed from the blood flowing through the kidney, so that their concentration in the renal vein is negligible. The above formula can then be simplified to $F = \frac{UV}{A}$, which will be recognized as the familiar clearance formula. Examples of such substances are diodrast and para-amino-hippurate (PAH), and their clearances at low plasma

concentrations are nearly equal to the renal plasma flow. In actual fact they are approximately 10 per cent. lower than the true plasma flow, and it is assumed that this 10 per cent. of blood is flowing through non-functioning renal tissues. The PAH or diodrast clearance is therefore known as the effective renal plasma flow (ERPF), and from this the renal blood flow can be calculated if the hæmatocrit is known. The ERPF in normal man is $697 \pm 135{\cdot}9$ ml./min.

Filtration Fraction. Knowing the glomerular filtration rate and effective renal plasma flow, it is possible to calculate the percentage of plasma water that is filtered at the glomerulus. This is called the filtration fraction (FF)

$$FF = \frac{GFR}{ERPF} \times 100$$

and is normally 19 ± 2 per cent. Characteristic alterations in the filtration fraction are found in certain diseases.

Tubule Function. Reabsorption : Certain substances such as glucose are reabsorbed from the glomerular filtrate, but the capacity for re-absorption is limited by a maximum. This maximum is known as the re-absorptive Tm (tubular maximum) for the substance concerned and is estimated as follows : The blood level of the substance, e.g., glucose, is raised considerably by a suitable infusion and its plasma concentration (P) determined. The glucose now appears in the urine and the amount excreted per minute (UV) and the glomerular filtration rate are determined. The amount filtered per minute is P $\times$ GFR (for the concentration in the glomerular capsule is the same as in the plasma) and the amount appearing in the bladder per minute is UV. The difference between UV and P $\times$ GFR is the amount being re-absorbed per minute. In the case of glucose this is known as the TmG. In normal man TmG is $375 \pm 79{\cdot}7$ mg./min.

Example :

Suppose GFR = 100 ml. per minute (determined as above).
P = 400 mg. per 100 ml. (plasma glucose concentration).
V = 5 ml. per minute (urine volume per minute).
U = 2,000 mg. per 100 ml. (urine glucose concentration).

The amount of glucose filtered per minute = 400 mg.
,, ,, appearing in urine per minute = 100 mg.
,, ,, being reabsorbed per minute = 300 mg.

Excretion : Other substances are excreted by a process of glomerular filtration and tubular secretion, e.g., diodrast and

para-amino-hippurate (PAH). The excretory Tm is determined in much the same way as for re-absorptive Tm. The plasma level of the substance is raised to a high level so as to cause the tubular excretory system to work at maximal rate (Fig. 11/7). The amount of the substance appearing in the urine per minute will be greater than the amount filtered at the glomerulus per minute. The difference is the amount excreted per minute by the tubules. The normal value for the tubular excretory maximum of diodrast (TmD) is 51·8 ± 8·73 mg. per minute. The normal value for the tubular excretory maximum of para-amino-hippurate (TmPAH) is 76 mg. per minute. The Tms, both re-absorptive and excretory, are proportional to the mass of functioning tubular tissue. When the kidneys become smaller or tubule functions diminish, the Tms fall. In the absence of any specific interference with the enzyme systems responsible for re-absorption of glucose or excretion of diodrast or para-amino-hippurate, the Tms for these substances fall to the same degree. Relative hyperæmia or ischæmia of the kidney can be assessed by comparing the Tm with the ERPF—e.g., a high ERPF/Tm ratio indicates relative hyperæmia, and a low ratio relative ischæmia of the kidney.

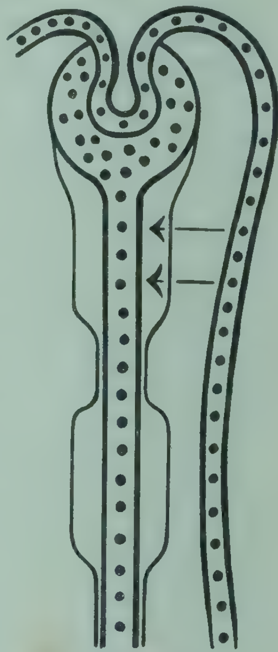

FIG. 11/7.
PAH high blood level.

Simple Tubule Function Tests. One of the functions of the tubules is to reabsorb water from the glomerular filtrate, so that the concentration of some substances is considerably higher in bladder urine than in the plasma. For instance, the concentration of creatinine in the bladder urine in dehydrated, and therefore oliguric, subjects may be 200 times greater than in the plasma. That is to say, the ratio

$$\frac{\text{urine concentration}}{\text{plasma concentration}} \quad \frac{U}{P} \quad \text{is } 200/1.$$

If no tubular re-absorption of water occurred the ratio would be 1/1. The lowest normal ratio for U/P creatinine in man is approximately 5/1 during extreme diuresis, 50/1 at a rate of urine flow of 2 ml./min., and 100/1 at a rate of flow of 1 ml./min. Low ratios

of U/P for creatinine, urea, inulin and certain other substances may indicate tubular dysfunction.

Another function of the tubules is to reabsorb sodium and chloride from the glomerular filtrate. Under conditions where the body lacks these ions the urine concentration of sodium and chloride falls to very low levels, so that the ratio $\frac{\text{plasma concentration}}{\text{urine concentration}}$ becomes very high. In certain conditions where there is tubular damage, these ratios may remain at approximately 1/1, despite the fact that the body requires these ions. The P/U ratio for sodium and chloride under these circumstances can be used as an indication of tubule dysfunction.

A very commonly used test in clinical practice is the concentration-dilution test. It is generally believed that a failure to pass concentrated urine represents a failure of water re-absorption, whilst failure to pass dilute urine indicates an inability of the tubules to reabsorb solids. If this were the case, this test would be a measure of tubule function. In all probability it is not. It is, nevertheless, a useful index of renal function as a whole. A probable explanation of the fixation of urine specific gravity, given by Platt (1951), is as follows : Osmotic diuretics cause the urine specific gravity to fall if previously high and to rise if previously low. Urea retention due to glomerular filtration defect causes a greater load of urea to be filtered per remaining nephron, so that the urea acts as an osmotic diuretic.

Examples of Disordered Renal Function in Disease

Idiopathic Essential Hypertension. Early in the course of the disease the renal blood-flow is reduced, but the glomerular filtration rate and tubular functions are normal. The filtration fraction is high and the ERPF/Tm ratios are low, indicating relative ischæmia. This ischæmia is due to tonic muscular spasm of the renal arterioles and can be abolished in certain ways ; structural organic narrowing of the vessels does not occur in the early stages. Later there is a further fall in blood-flow and the glomerular filtration rate and tubular maxima become reduced, indicating that organic change and loss of nephrons has developed.

Cardiac Failure. All types of cardiac failure, including both high and low output types, are associated with a fall in glomerular filtration rate and effective renal plasma flow. The filtration fraction is very high and the ERPF/Tm ratios low, indicating renal ischæmia, occurring as the result of increased arteriolar tone

in the kidney and brought about by a humoral mechanism. In addition to these changes in function there is an increased tubular re-absorption of water and salt. This, combined with the reduction in glomerular filtration rate, plays an important *rôle* in the genesis of œdema in cardiac failure (see Chapter VIII, p. 168).

Glycosuria. Consideration of the mechanism of glucose recovery by the tubules reveals that glycosuria could result in three ways :

(*a*) If the GFR and TmG are normal and the plasma level of glucose is raised, glycosuria will result because the filtered load of glucose presented to the tubule is greater than the re-absorptive capacity—for example, diabetes mellitus.

(*b*) If the plasma level of glucose and TmG are normal and the rate of glomerular filtration is considerably increased, glycosuria will result, for once again the filtered load is higher than the TmG. This occasionally occurs in normal subjects after massive infusions of plasma which raise the glomerular filtration rate.

(*c*) If the plasma glucose level and the GFR remain normal and the TmG is reduced, even a normal filtered load will exceed the TmG and glucosuria will result. This occurs after phloridzin poisoning and is the mechanism in benign renal glycosuria. In the latter instance, there is a congenital disturbance of the enzyme system responsible for glucose re-absorption. A number of other isolated tubule function defects is known.

The physiology of the kidney, the theoretical basis for renal function tests, and descriptions of disordered patterns of function in disease are fully reviewed by Homer Smith (1951).

G. M. BULL.

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ANURIA, OLIGURIA AND ACUTE TUBULAR NECROSIS

In recent years our whole approach to renal pathology has been undergoing a change dictated by the increasing knowledge of renal physiology. We can now view the kidney quantitatively as a functioning unit and by means of histo-chemical techniques

even piece together a few of the finer details of the metabolism of its cells. This new knowledge of renal function and metabolism is beginning to throw light into some of the darker corners of renal pathology. By way of illustration we propose to consider a group of renal diseases, characterized by anuria or oliguria, the intensive study of which has in recent years diverted the trend of pathological thought from the purely morbid anatomical towards a more balanced approach.

Anuria, the complete cessation of urinary flow, or oliguria, its reduction to pathologically small quantities, are evidence of gross physiological disturbances in the nephrons and may be due to a number of widely differing causes, some obvious, others obscure. In some cases the oliguria or anuria results from obstruction to the urinary tract and its cause is abundantly clear; in others gross organic changes in the kidney itself—such as those of glomerulo-nephritis, hypertension, infections and numerous other conditions—are responsible. Excluding these grosser lesions, we are left with a residue of diseases in which anuria or oliguria is present, and death may occur in uræmia, yet the purely morbid anatomical changes in the kidney may be slight. Into this class we may put the uræmia which may follow severe surgical operations or shock, burns, severe dehydration, abortion, blackwater fever, incompatible blood transfusion, the crush syndrome, the uræmia produced by certain poisons, and so on. In this group, pathological changes when present in the kidney are essentially tubular. At first sight it would seem that this varied catalogue of diseases has little in common, but on further analysis two main classes emerge: Firstly, there is a group in which the cause is obviously a poisoning of the kidney; for example, the anuria following ingestion of a mercurial salt or carbon tetrachloride. In poisoning the kidney is not infrequently far more severely affected than other parts of the body. This may be the result of water re-absorption in the tubules during excretion of the poison, the concentration of the poison becoming higher in the tubules than anywhere else in the body, except possibly at the portal of entry. It is not surprising, therefore, that in these cases when obvious histological changes are found they are usually confined to the tubules. Secondly, there is a far larger group of apparently diverse states which give rise to anuria. When these are considered from the functional point of view, it becomes clear that they are invariably associated with a disturbance of the general circulation, which may be the result of oligæmic "shock," hæmor-

FIG. 11/8. Necrosis of the nose in a patient suffering from an extreme degree of congestive cardiac failure. Illustrating the tissue damage caused by extreme vaso-constriction in certain fields of the circulation that accompanies general circulatory failure. This patient also developed gangrene of the fingers and severe renal damage.

[*To face page* 283.

rhage, dehydration, severe hypotension, or a reflex or humoral vaso-constriction in certain parts of the circulation. During recent years an imposing and convincing body of evidence has accumulated which makes it reasonably certain that under all these conditions there is a severe degree of renal vaso-constriction, amounting at times to a virtual cessation of renal blood-flow (Van Slyke, 1948; Homer Smith, 1951). It would seem that when the general circulation becomes inadequate the blood-flow to large areas of the body, including the kidney, is reduced, presumably in order to maintain the circulation in more vital areas such as the brain, and the reduction to the less vital parts may be extreme, so that functional or even organic changes may be found in them (Fig. 11/8). In the case of the kidney, these changes lead to a state which has been called " extra-renal azotæmia " or " extra-renal uræmia," names which are not altogether apt, for the azotæmia or uræmia is due to diminished renal function just as much as in diseases where there is an obvious anatomical renal abnormality. In most of these general circulatory disturbances this rearrangement of the circulation with renal ischæmia is not severe enough, or does not persist long enough, to produce obvious histological changes. In others, renal lesions are produced and are invariably found in the tubules. This is not surprising, for the tubule cells are metabolically very active and have a relatively high oxygen consumption as compared with the renal stroma, blood-vessels, etc. When the amount of anatomical renal damage is minimal, restoration of the general circulation leads to prompt recovery of renal blood-flow and with it a disappearance of the azotæmia, but when severe renal damage and particularly necrosis of tubule cells has been produced, renal function is slow to recover and may never return to normal. Thus tubular damage may be produced in two main ways, first by the action of a poison and secondly by ischæmia. Not infrequently both factors operate, and it is then difficult to decide which played the major *rôle*.

Nomenclature. The term " tubular nephritis " has had a chequered history. The older text-books of pathology describe the condition with confidence, but with an increasing understanding of the anatomy of intra-capillary glomerulo-nephritis, acute tubular nephritis has disappeared as a form of Bright's disease, though some mention is usually made of tubular degenerative changes, sometimes amounting to necrosis, associated with febrile and toxic states, acute infections, metallic poisoning and the like. The tendency to regard tubular necrosis as a transitory

and generally terminal change has been checked by certain experimental observations such as those of Duguid (1936), which show that a tubular lesion of sufficient severity may lead to renal sclerosis with hypertension, cardiac hypertrophy and uræmia. In more current terminology, the term "nephrosis" has crept in for any non-inflammatory tubular lesion. This term has been used in so broad a sense, and its significance altered so greatly, that it has almost lost its meaning in classification. Latterly, in this group, the expression "lower nephron nephrosis" has been used by Lucké (1946) to describe a tubular lesion involving especially the ascending limb of Henle's loop and the second convoluted and collecting tubules. This lesion was noted in septic abortion by Bratton (1941) and described in detail by Bywaters and Dible (1942) in traumatic anuria ("the crush syndrome").

"Lower nephron nephrosis" is not a happily chosen term, as there is evidence that in the conditions in which it has been used, neither the anatomical nor the functional lesions are limited to one portion of the nephron, although the more striking histological changes may certainly be seen in the lower reaches of the renal units. We feel that a more satisfactory term is "acute tubular necrosis." In all the conditions being now considered, death of tubule cells is the cytological hall-mark. In the very mildest form, cellular dysfunction rather than death may predominate, but there is nevertheless an increased rate of cell death, as evidenced by an increase in the numbers of tubule cells found in the urinary sediment.

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J. H. DIBLE.

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ACUTE TUBULAR NECROSIS[1]

Acute tubular necrosis is a consequence of a very wide variety of renal insults and is associated with a characteristic clinical

[1] Data on the clinical and functional picture in acute tubular necrosis, and the methods of treatment were obtained with Drs. A. M. Joekes and K. G. Lowe at Hammersmith Hospital from 1947 to 1951.

picture, anuria or severe oliguria being the most prominent sign. Over sixty "causes" have been recognized (Darmady, 1950), but these can conveniently be considered in three main groups :

1. Those due to the direct action of poisons on the renal epithelium—poisoning with mercury, carbon tetrachloride, phenols, etc.

2. Those due to renal ischæmia—the "shock kidney" (Van Slyke, 1948), and the anuria following concealed accidental hæmorrhage, etc.

3. Those of uncertain or mixed ætiology—crush syndrome, criminal abortion, blackwater fever, incompatible blood transfusion, etc. In almost all the examples of this group, both toxic and ischæmic factors probably play a part.

Clinical Course. This can be conveniently divided into four phases :

The onset phase, during which the causal agent is operating, and the kidney is damaged, perhaps temporarily, possibly irreparably. During this phase the urine volume falls. Where severe poisoning is the cause, this phase passes inevitably into the succeeding phases. Where renal ischæmia is responsible and is not severe, or is relieved soon after its development, almost immediate and complete recovery will follow. A short period of oliguria or anuria and an increase in the number of renal cells in the urinary deposit may be the whole picture. Abortive attacks of this type, the so-called extra-renal azotæmias (Fishberg, 1939) are extremely frequent. When the renal ischæmia is more severe or persists for some considerable time (probably at least three hours of very severe renal ischæmia), the rapidity of renal recovery is lessened, oliguria or anuria may persist for longer and longer periods, and the patient passes into

The second phase of established oliguria or anuria, which may last for periods of up to three weeks or occasionally longer. There appears to be an almost continuous spectrum of severity from the mildest, with minimal oliguria, to the most severe with long-standing oliguria or anuria, but in the majority of patients this phase lasts from ten to fourteen days. During this time the blood urea mounts progressively, but in most patients in whom treatment has been correct (see later), very little general disturbance of health is evident. Where treatment is incorrect, symptoms and signs of uræmia develop and the patient may die. This phase is terminated by an increase in urine flow which heralds

The third or early diuretic phase, in which the urine volume increases until it exceeds more than a litre a day. Occasionally the increase in urinary volume is abrupt. This phase is characterized by very poor tubule function and, in the absence of adequate treatment, a variety of disturbances of electrolyte and water balance may occur (see later).

The fourth or late diuretic phase follows and is characterized by returning tubule function.

Death may occur in any of the first three phases. During the

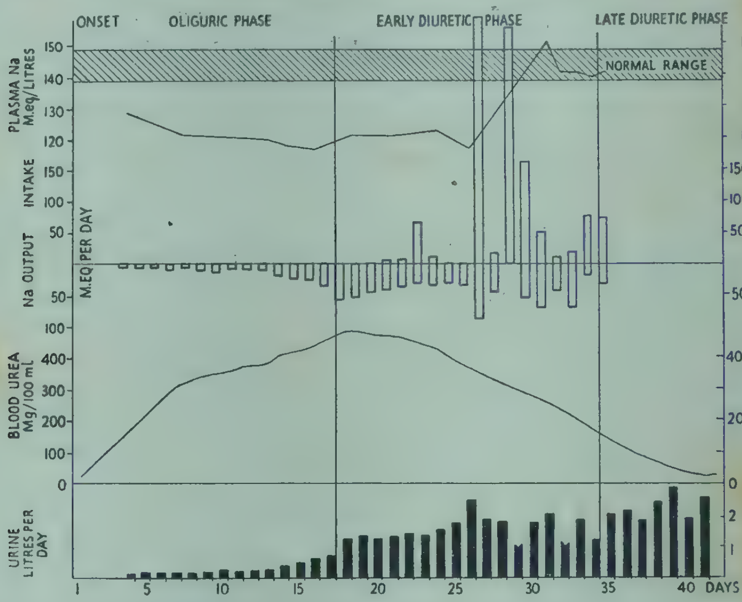

FIG. 11/9. Plasma sodium levels, intakes and urinary outputs of sodium, blood urea levels and urine volumes during the course of acute tubular necrosis following an abortion. Between the onset and day 26 the patient was on a mineral-free diet and, despite the low plasma level of sodium, considerable quantities were lost in the urine.

first, and often during the succeeding two phases, it is due not to the renal damage, but to the disease or original insult which caused this. During the oliguric or anuric and the early diuretic phases it may result from the renal dysfunction alone. Fig. 11/9 illustrates the phases in a typical case.

Tubule Function. Whatever the primary cause of acute tubular necrosis, the pattern of disturbed function is the same (Bull, Joekes and Lowe, 1950). In tubular necrosis of all sorts there is a general disturbance of function affecting all parts of the tubule. It can be demonstrated in the oliguric or anuric phase

and in the early diuretic phase. During this time the urine specific gravity remains fixed at approximately 1.010 and the ratio of urine concentration/plasma concentration of creatinine and urea are low, clearly indicating a severe degree of failure of the tubule to reabsorb water (see Fig. 11/10).

The ratios of plasma concentrations/urine concentrations of chloride and sodium are also low, even when the body urgently needs these ions. This indicates an inability of the tubule to

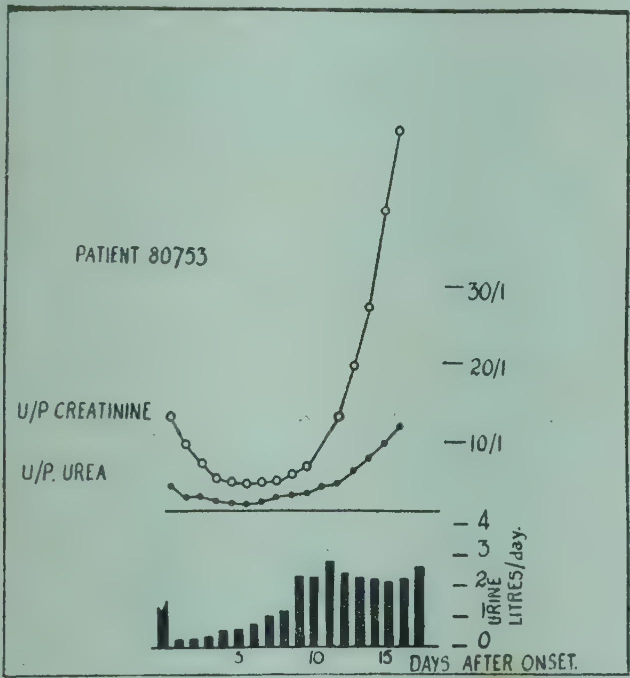

FIG. 11/10. The ratios of urine/plasma concentrations of creatinine and urea in a patient suffering from acute tubular necrosis following a hæmolytic transfusion accident. The low values of the ratios before day 14 indicate tubular dysfunction (*Clin. Sci.*, 1950, **9**, 379).

reabsorb electrolytes from the glomerular filtrate (see Fig. 11/11). This particular disturbance of tubule function has an important bearing on treatment, for there may be a considerable and uncontrolled loss of electrolytes in the urine in the early diuretic phase (see Fig. 11/9). The tubule function is as grossly disturbed during the oliguric phase as in the early diuretic, but the quantities of electrolyte lost at this time are of much less importance because of the low rate of urine flow. In addition to sodium and chloride loss in this way there may be excessive loss of potassium, causing

a lowering of the serum potassium concentration to the level at which paralysis, in every way similar to that occurring in family periodic paralysis, may occur. Fortunately the disturbance of tubule function is so uniform in all cases that, within fairly narrow

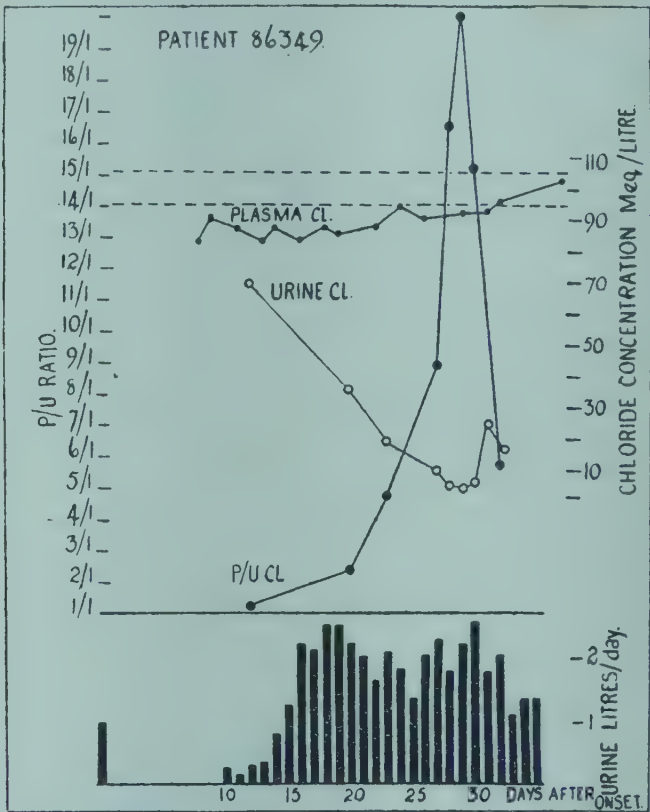

FIG. 11/11. The ratio of plasma/urine concentration of chloride in a patient suffering from acute tubular necrosis following mercury poisoning. The low ratios up to day 26 (approx.) indicate considerable tubule dysfunction. The plasma chloride concentration was sub-normal, yet the urine chloride concentration was high. The fall of the ratio after day 28 does not indicate a return of tubule dysfunction, but merely that the body needs for chloride have been met (*Clin. Sci.*, 1950, **9**, 379).

limits, the plasma/urine ratios for sodium and chloride are about 2/1 in the oliguric and early diuretic phases. In other words, the urine passed contains approximately the same quantity of sodium and chloride as an equal volume of half physiologically normal saline. This considerably simplifies replacement therapy (see later). All other tubule functions which have been examined show similar degrees of disturbance ; Sirota (1949) found very

FIG. 11/12. A radio-opaque catheter in the renal vein. Through the catheter blood can be sampled for analysis.

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low values for TmPAH; Bull and his co-workers (1950) found very low values for TmG, and all these workers report very low extraction ratios for PAH at the same time as other tubule functions had been shown to be disturbed. In treating patients with acute tubular necrosis it is convenient to know how long the tubule function is likely to remain seriously affected. On

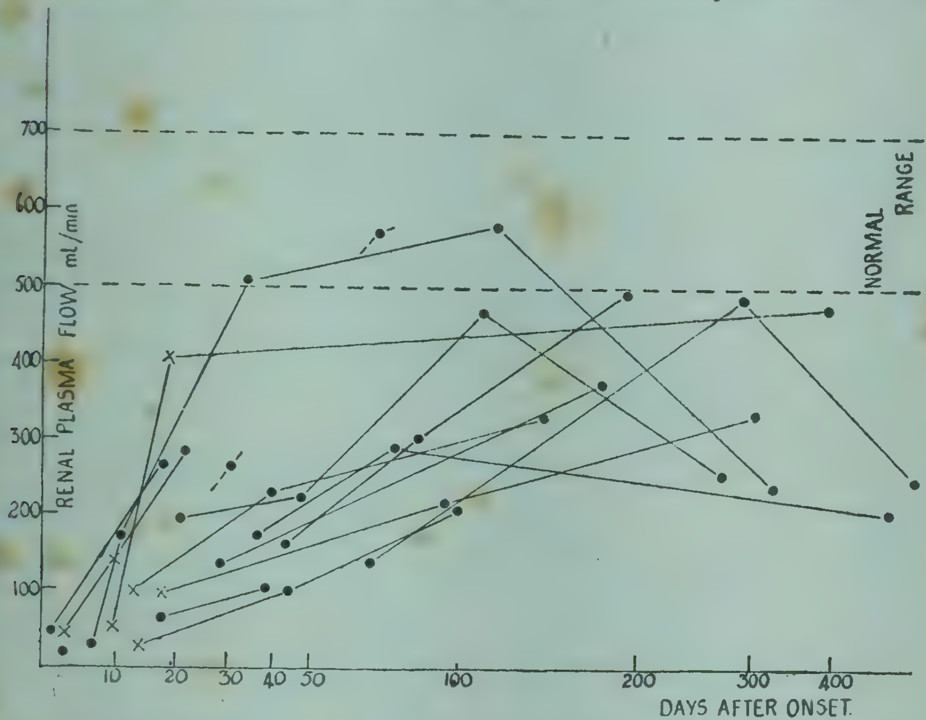

FIG. 11/13. The renal plasma flow in acute tubular necrosis. Note the very low values in the early part of the illness (*Clin. Sci.*, 1950, 9, 379).

an average, the early diuretic phase is equal in duration to the preceding anuric or oliguric phase (Bull *et al.*, 1950). For example, if a urine flow of over 1 litre is achieved on the twelfth day of the illness, tubule function is likely to remain seriously impaired until the twenty-fourth day.

Renal Blood-flow. Because of the poor tubule function in acute tubular necrosis the simple method of estimating renal blood-flow by measuring the renal clearance of para-amino-hippurate or diodrast at low blood concentrations is invalid. The rate of renal blood-flow can, however, be determined by the method described on p. 277. This entails the simultaneous sampling of blood from an artery and from the renal vein, and the

collection of urine over a timed period. Blood from the renal vein is obtained by passing a radio-opaque catheter through the antecubital vein, right auricle and inferior vena cava into the renal vein (see Fig. 11/12) (Warren *et al.*, 1944). Using this technique (Sirota, 1949 ; Bull *et al.*, 1950), it has been found that the renal blood-flow in the oliguric phase is extremely low, sometimes below 5 per cent. of normal ; it then rises slowly over a period of weeks, but very seldom reaches normal levels even after months. The degree of reduction of renal blood-flow after three to six months is seldom severe. Fig. 11/13 shows the rates of renal blood-flow in a series of patients suffering from acute tubular necrosis. At the same time as samples of blood are obtained for estimation of arterial and renal vein concentrations of substances, further samples can be taken for estimating the oxygen content. These show that the renal vein blood is more venous in character than normal, i.e., has a lower than normal content of oxygen. This suggests that the rate of renal blood-flow is lower than normal, and is evidence against any appreciable diversion of renal blood-flow through arterio-venous anastomoses or juxta-medullary regions, as suggested by Trueta and his colleagues (1947).

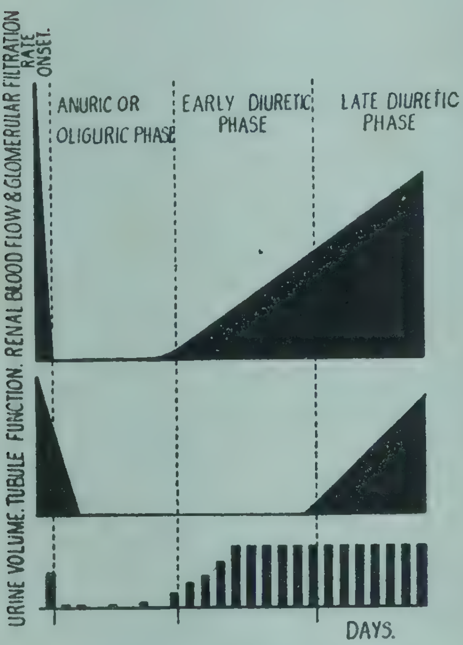

FIG. 11/14. Diagrammatic representation of the functional disturbances in acute tubular necrosis (*Clin. Sci.*, 1950, **9**, 379).

Glomerular Filtration Rate. The ordinary methods for glomerular filtration determination may not be valid in acute tubular necrosis, because there may be a passive diffusion of the test substance through damaged tubular walls. It is, however, certain that the rate of glomerular filtration must also be very low because the renal blood-flow is so low. The overall pattern of disturbed renal function in acute tubular necrosis is shown diagrammatically in Fig. 11/14.

G. M. BULL.

Morbid Anatomy

The ideal of the pathologist in any case of disease is to be able to portray the whole history of the lesion and to correlate morbid anatomical changes with functional alterations. He is, however, always at a disadvantage in dealing with human material in that death does not occur until the changes have reached a certain degree of severity and therefore, for information upon the earliest lesions, he is indebted to the chance concurrence of some other fatal condition which brings the patient to autopsy. Even in these cases there must always remain, at the back of his mind, some lingering doubt as to whether the early lesions which he sees and interprets as the forerunners of a later, more often seen, and fatal stage of the disease, would in fact have evolved into this condition had the patient survived longer. In no organs are these considerations more weighty than in the kidney, where there is an added trap for the unwary in that autolytic changes can occur very quickly, so that much of the scanty material which comes to hand has to be discarded since a delay in fixation of forty-eight hours or so is by no means rare. With these provisos we shall endeavour to give a description of the evolution of the renal lesions in our cases of tubular anuria. The study is a personal one based upon the detailed examination of kidneys from 62 cases of anuria in which clinical histories were available.

From the pathological point of view, cases of tubular anuria seem to fall into two main groups :

A. *Those in which there is gross necrosis of tubular epithelium with a maximum incidence of this in the first convoluted tubules.* Cases of this sort are often due to simple chemical poisons, such as mercuric chloride, carbon tetrachloride, methyl bromide, etc.

B. *Those in which there is a selective damage of the nephron, the grosser visible changes being found more especially in the boundary zone and in the second convoluted and collecting tubules.* Most of the cases in the ætiological group 3 (p. 285) fall into this class, such as the anuric kidneys of incompatible blood transfusion, those associated with gross intravascular hæmolysis, extensive muscular damage—the " crush syndrome " or " traumatic anuria." Lucké's review (1946) of these lesions and his reference to the condition as " lower nephron nephrosis " is discussed on p. 284.

It remains to be said that the status of many other anurias, including many in the clinical group 2, is still *sub judice.* The lesions are essentially tubular and rarely cause fatal uræmia.

Such are the changes associated with prolonged anoxia from "shock," hæmorrhage, burns, sulphonamide hypersensitivity, and a number of other conditions. Our limited experience leads us to place them with the class of case we are considering now. We have also encountered lesions which, in part but not wholly, reproduce this picture in quite a variety of pathological conditions involving the kidney.

Gross Necrosis of Tubular Epithelium. The changes are shown in Fig. 11/15 which is from a case of nembutal poisoning [1] with anuria of forty-eight hours' duration. The almost complete necrosis of the cells of the first convoluted tubule is the striking change observed. The epithelial cells are swollen, the lumina obliterated ; by contrast the thin portions of the loop and the ascending limb of Henle and the second convoluted tubules appear relatively normal though collapsed. Their nuclei are a little pyknotic and these portions of the nephron show in strong relief in the cortex against the mass of necrotic proximal tubules. The cortex appears generally vascular and no lesion can be detected in the glomeruli and vessels. There are no casts, no pigment and no evidence of inflammatory interstitial reaction. In some cases there is considerable desquamation of the necrotic epithelial cells and a good deal of granular débris is seen filling the tubules, but in others the cells still adhere to their basement membrane. There is also some necrosis, desquamation, and heaping up of desquamated cells in the collecting tubules and ducts of Bellini. We have no exact knowledge of what the appearances may be in recovery, but where this occurs we assume that there has been less complete necrosis of the epithelial cells, desquamation of the necrotic elements and regeneration of epithelium by the remaining viable cells.

What is the explanation of the anuria associated with this lesion ? The histological impression is that no urine is passing down the tubules. There appear to be two theoretical possibilities. Firstly, the complete ablation of all function of the cells of the first convoluted tubules with rapid and complete reabsorption of the filtrate into the renal capillaries and venules. Such a mechanism would not be expected to be detectable by any ordinary histological examination. A second possibility is a swelling of the renal tissue due to the osmotic absorption of water, resulting from the breaking down of the protoplasm of the necrotic cells and causing general compression of the renal capillaries and veins leading to gross ischæmia of the organ. Such a mechanism may be of local significance in the selective type of tubular necrosis, which we shall discuss shortly. If

[1] This particular form of poisoning is not a usual cause of the lesion but the tissues in the present case were so well preserved and the clinical history so clear that we have used it in illustration.

present. it might be expected to be evidenced by histological changes in the lower portions of the nephron or by evidence of œdema or circulatory disturbance in the kidney, but these are in our experience not found in the cases now under consideration.

Selective Necrosis of Tubular Epithelium. This is the group to which the term "lower nephron nephrosis" has been applied. It contains the larger proportion of fatal cases of anuria, in which selective epithelial necrosis with a maximum incidence on the second convoluted tubule is the outstanding lesion. Of a gross total of 62 cases on which our experience is based, 40 showed lesions bringing them into this group. In 36 of these the lesion was sufficiently well developed to be regarded as characteristic, whilst in the remaining four the changes were considered to be early and in process of development. The clinical ætiology of the 40 cases is :

Incompatible transfusion	11
Crushing injury	11
Crush and transfusion	4
Operation "shock" and transfusion	4
Blackwater fever	2
Burns	2
Criminal abortion	2
Sulphonamide reaction	2
Quinine poisoning	1
Hæmolytic anæmia and operation "shock"	1

The high proportion of examples of crushing injury is due to the inclusion in the series of the cases collected by Bywaters and Dible (1942). It is often difficult, if not impossible, to be sure of the exact part played by a number of possible ætiological factors, since the victim of a serious accident may well have suffered from shock, with the aggravation of an operation under anæsthesia, and have been treated by sulphonamides and blood transfusion ; whilst a patient upon whom an heroic operation has been performed, and who has been severely shocked, is certain to have had blood transfusions, about the compatibility of which we may often be left in doubt.

It should be said at the beginning of a description of the pathology of renal tubular necrosis that in its later stages it is usually associated with inflammatory and reactive changes which may dominate the picture. These are not the cause of the anuria, but develop subsequently. In the same way,

in the "pigment nephroses" the association of pigment with casts and necrotic cells is doubtless a secondary event, except in so far as pigment may be of itself toxic and aggravate a change already under way. From the clinical angle it would seem that the onset of anuria in many of these cases must be an abrupt event. Pain in the loin may occur during the currency of an incompatible blood transfusion, which seems to indicate a sudden renal damage. It is followed at once by anuria, which can hardly at this stage be due to the development of renal tubular necrosis, and must therefore be regarded as an event secondary to vascular alterations. The kidneys we have been able to examine for early lesions, and on which our conception of the evolution of the process is based, have been obtained from patients who have received and died from severe crushing injuries and in whom every probability existed that uræmia with the full "crush syndrome" would have developed had the patients survived long enough. Such an assumption is plainly open to criticism, but it seems inescapable if we are to look for the earliest renal changes. An assumption of a preliminary circulatory change leading on to secondary renal tubular necrosis thus dominates our conception of the evolution of the histological changes, although it cannot affect the facts. Such a view is borne out by the finding that in the earliest cases renal parenchymal lesions are essentially absent. It may be recalled here that in an experimental investigation on the effect of transient complete renal ischæmia in rabbits Scarff and Keele (1943) do not describe tubular lesions earlier than the second day after the period of ischæmia.

Development of the Lesions. In agreement with the observations of Scarff and Keele, the earliest histological changes we have been able to detect have been found after thirty to forty hours' anuria. This change (Fig. 11/16), which we may call the *first stage*, is a limited necrosis of the first convoluted tubule in its descending portion (descending limb of Henle) at the boundary between the cortex and medulla. The case illustrated was one of severe burns with forty-two hours' survival during which anuria was complete. An almost exactly similar picture was found in a patient who survived gastrectomy for thirty-six hours in a state of profound "shock." As far as histological evidence goes the glomeruli play no part in this process, they are generally congested at this and any earlier stage, and, in fact, the kidneys at this period appear full of blood, though this is not the case later on when the cortices of many kidneys appear anæmic. About this time—from thirty hours onward—with some variation from case to case, cast material appears in some of the thin Henle tubules in the medulla and some of the second convoluted tubules in the cortex. These casts are

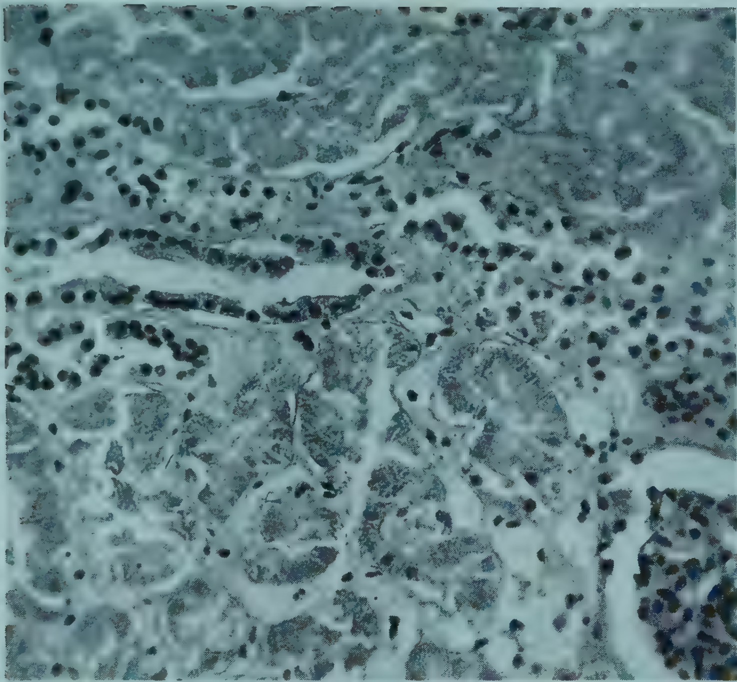

FIG. 11/15. Massive first convoluted tubular necrosis in nembutal poisoning. Forty-eight hours' duration. × 240. (*Case of Professor G. Hadfield.*)

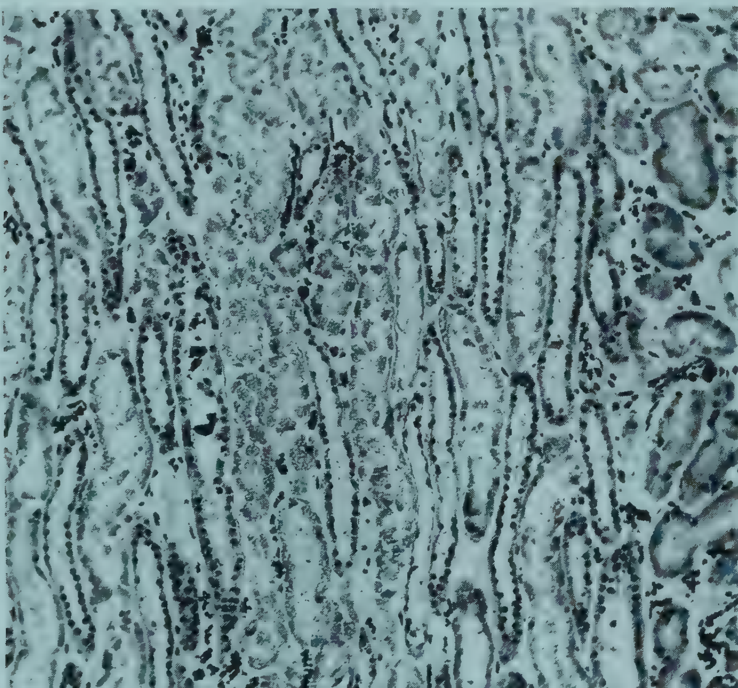

FIG. 11/16. Ascending and descending loops of Henle, showing selective necrosis of the cells of the latter. From a case of burns. Forty-two hours' duration. × 112. (*Case of Dr. Janet Vaughan.*)

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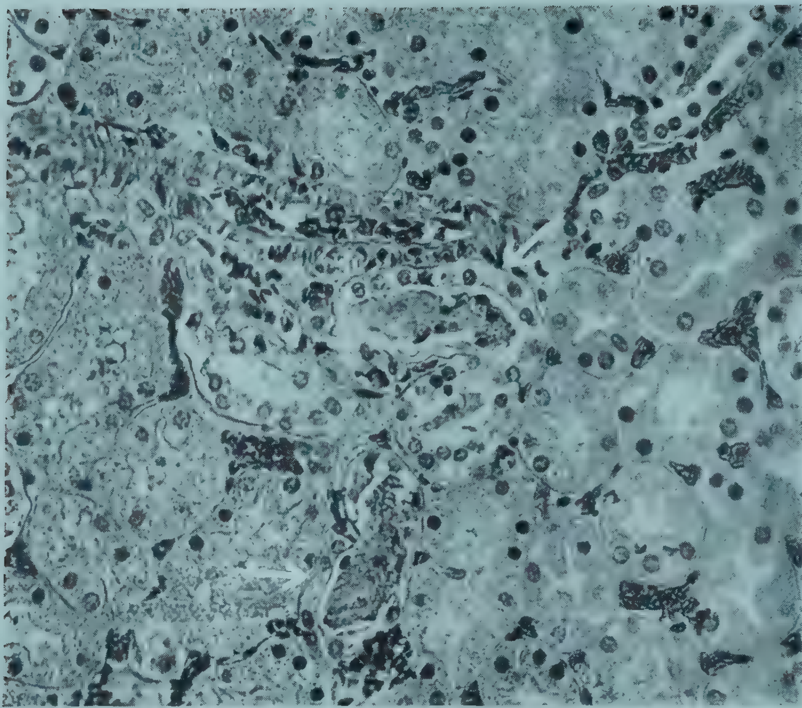


FIG. 11/17. Arrows indicate early unpigmented casts in the second convoluted tubules in the cortex. There is evidence of some pyknosis and cell desquamation in these tubules also. The glomeruli were congested. (*Case of Dr. E. G. L. Bywaters.*) Crush injuries with twenty hours' survival. × 235.

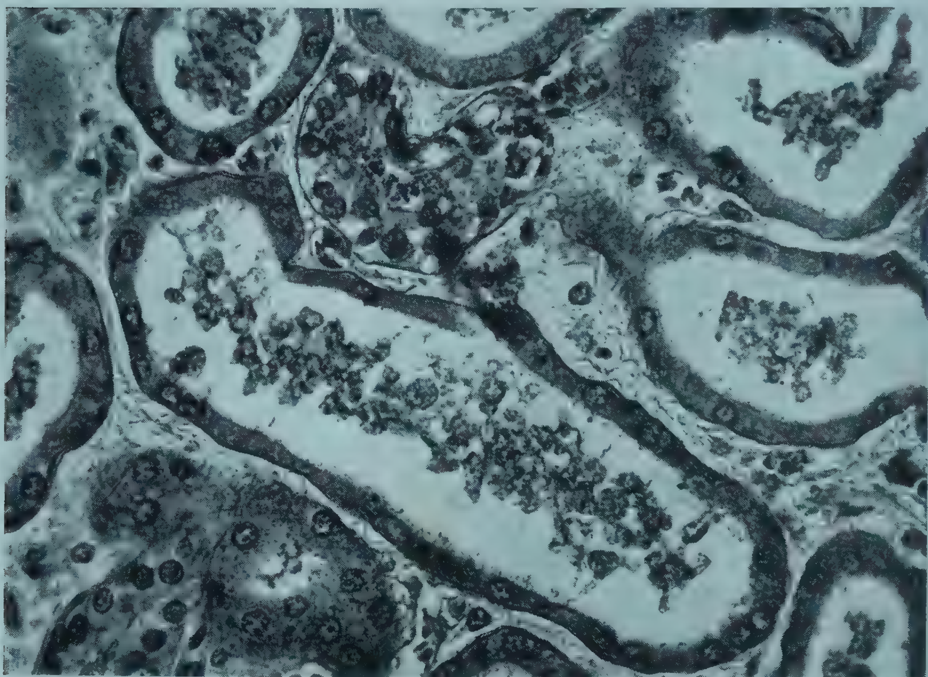


FIG. 11/18. First convoluted tubules showing dilatation, some flattening of the epithelium and the presence of cellular and amorphous débris. A single second convoluted tubule is also seen in the middle of the upper part of field containing a pigmented cast. Case of crush injury, seven days' duration. (Bywaters and Dible, *J. Path. and Bact.*, 1942, **54**, 111.) × 335.

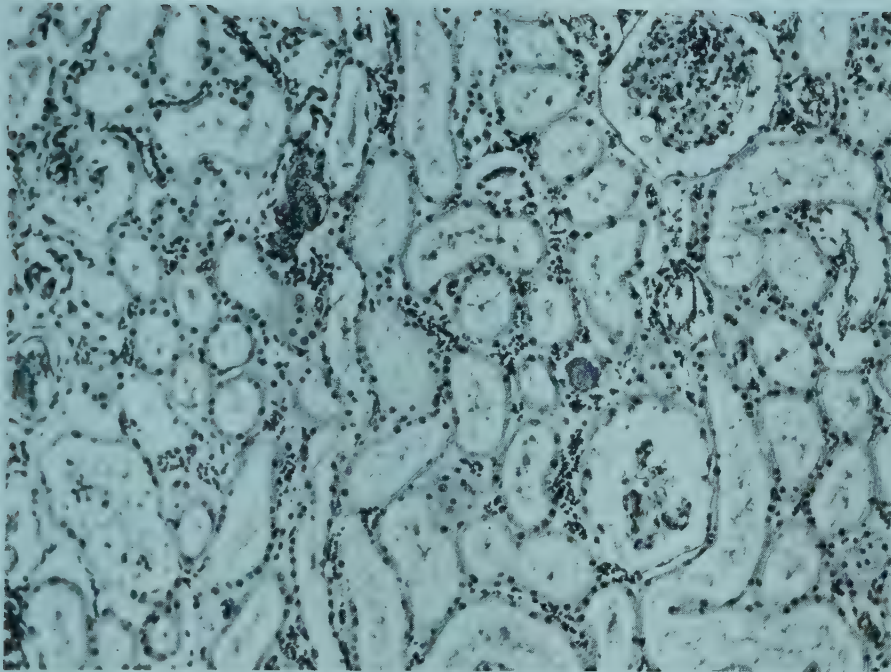


FIG. 11/19. Dilated first convoluted tubules. (*Case of Dr. de Nevasquez.*) Incompatible blood transfusion and anuria of three days' duration. × 112.

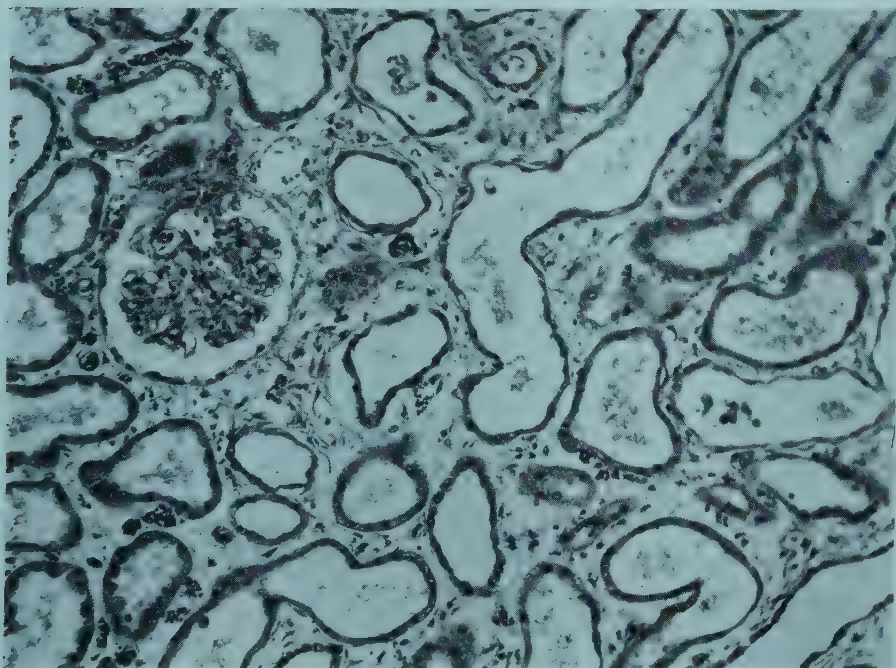


FIG. 11/20. Dilated first convoluted tubules. Case of incompatible blood transfusion and anuria. Nineteen days' survival on Bull's regime. Œdema of the cortex is also noteworthy. × 112.

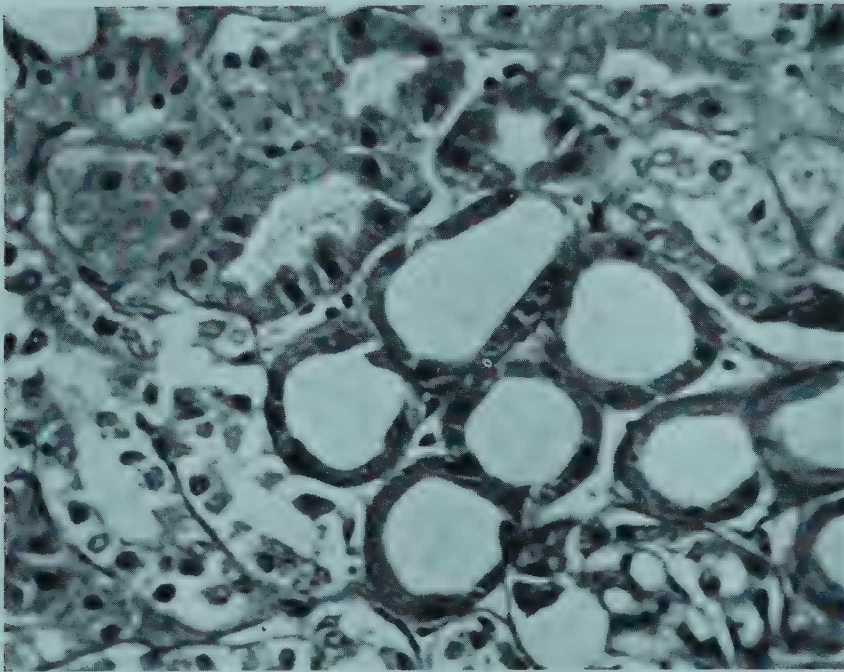


FIG. 11/21. Dilated first convoluted tubules produced by distending them with injection fluid. A portion of a glomerulus is shown at the right-hand lower corner, and above it the dilated tubules. On the left, unaltered first convoluted tubules are seen above and the normal second convoluted tubules below. × 300.

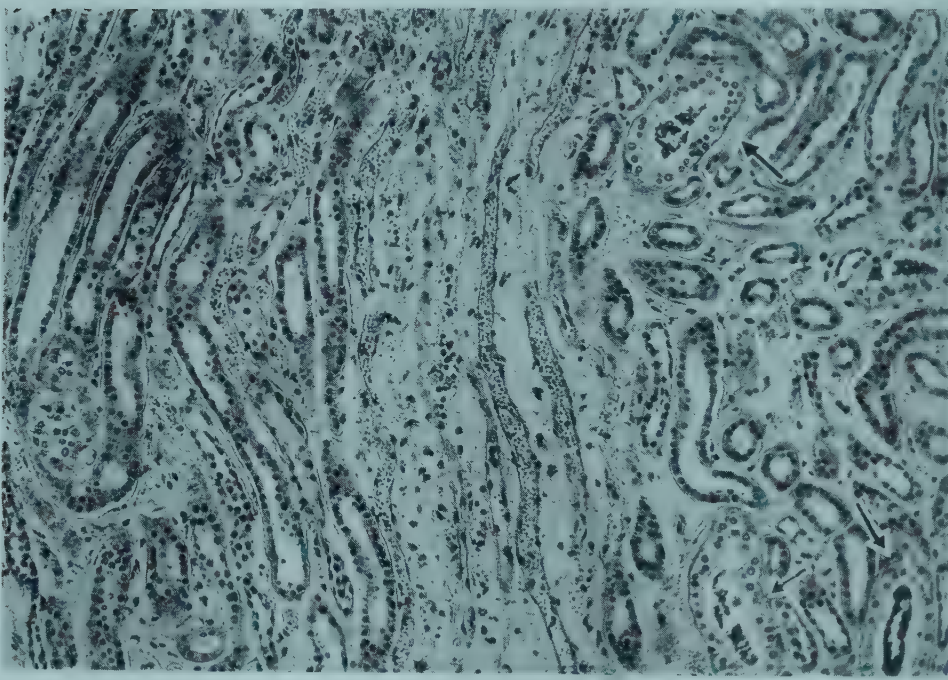


FIG. 11/22. Œdema of the boundary zone. Pigment casts are present in some of the collecting tubules—indicated by arrows. From a case of crush anuria of two days' duration. × 110.

at first hyaline and stain pink with eosin (Fig. 11/17). From this stage cell necrosis and catarrhal desquamative changes are becoming increasingly evident throughout the whole nephron, though so far they are not extreme nor are they always obvious upon casual examination. A predominance of these changes in the ascending limb of Henle and the second convoluted tubules also begins to be obvious. The glomeruli tend now to be less vascular than in the early cases, though there is the widest variation in this respect and their capillaries are quite permeable to injection. The condition of the first convoluted tubules varies considerably ; sometimes their epithelium is swollen and the lumina reduced; sometimes their epithelium is flattened and the tubules are dilated. Apart from the fact that a normal appearance is seen in the very early cases, no significant relationship between the duration of the condition and the state of these tubules in this respect can be made out. It is usual, however, for them to contain considerable amounts of débris (Fig. 11/18) which appears to represent, in part at any rate, the degenerate remains of desquamated epithelial cells. These changes have been found to persist, with evidence of desquamation and regeneration, throughout the duration of the disease covered by our observations. We may add that McManus (1950) has found a loss of alkaline phosphatase from these tubule cells.

The significance of dilatation of the first convoluted tubules which is sometimes a marked feature of the picture is a matter of great interest. One is tempted to regard it as a result of continued catarrhal desquamation and the replacement of the normal tall cubical epithelium by a debased substitute of less functional capacity. If this be the case the appearance might be expected when death is delayed for a long period, a fact not borne out by our observations, there being no constant association of these conditions (Figs. 11/19 and 11/20). On the other hand, we have seen the same appearances in ureteric obstruction from carcinoma of the cervix associated with widespread thrombi in the intra-renal veins and inflammatory lesions of the type presently to be described in the medulla and boundary zone. Further, a similar picture is easily produced by forcing injection material through Bowman's capsule into the lumina of the first convoluted tubules. This distends the tubule and flattens its epithelium (Fig. 11/21) and it would appear that whether epithelial regeneration is going on or not this picture indicates a distension of the tubules. If this interpretation be correct, we must conclude that urinary secretion is active and that filtrate is finding its way into the first convoluted tubules, although little or none is emerging from the ducts of Bellini. The first convoluted tubules, especially in cases of long survival, occasionally show an extreme ballooning, as described by Govan and MacGillivray (1950), and similar to that caused by sucrose infusion. If this be the explanation, and the condition is due to excess of sucrose or glucose in the cells, the observation is of importance as indicating some ability to function on the part of the first convoluted tubules in patients who die of uræmia and anuria.

Up to now our positive findings indicate a selective necrosis of tubules in the boundary zone of the cortex and evidence of

stagnation in the second convoluted tubules of the nephron and beyond. One is tempted to speculate that the effect of breakdown of the protoplasm of these cells may create a local zone of increased osmotic tension which may obstruct the passage of filtrate and restrict the circulation in the local veins. That there is positive evidence of vascular stagnation is certain (p. 300). There is also support for the view of an intrarenal disorder of tissue fluid in the development of œdema which may even be fibrinous. This is often prominent at quite an early stage in the process (Fig. 11/22), and is especially marked in the boundary zone and about the upper parts of the vasa recta, as these vessels detach themselves from the cortex and descend into the medulla, and in the medulla itself. This intermediate zone of medullary œdema need not be, and in the case illustrated here was not, associated with œdema of the cortex.

The significance of the cortical œdema, which is sometimes quite striking (Fig. 11/20) is not easy to assess. The mean survival time of the whole series was 6·2 days, the 17 cases showing cortical œdema survived on an average for 8·3 days and the 25 cases showing no cortical œdema had a mean survival time of 4·5 days. One might be tempted to relate this correlation of œdema with length of survival to an outflow of filtrate into the connective tissues of the cortex, but the matter is complicated by the relationship of this cortical œdema to general bodily œdema which is especially likely to be found where there has been a prolonged survival, and perhaps with this an over-enthusiastic administration of fluids. Exact information is lacking in this respect, but it may be said that of 10 cases in which general anasarca was noted in the post-mortem reports, 8 showed œdema of the cortex, which is more than a chance association. We conclude, therefore, that these two tend to be associated, though not absolutely.

Pigment Casts and Changes in the Boundary Zone. We must here record something of a hiatus in our knowledge. From this stage onwards all the cases we have followed to post-mortem merge into the class of "pigment nephroses," in that the casts which become more and more prominent in the histological picture become to an increasing degree pigmented. To what extent this is a constant occurrence in the development of the lesion irrespective of its mode of initiation, we are uncertain. All that we can say is that the casts in all the cases surviving over a certain time are more or less pigmented. This may be related both to duration and to the amount of pigment available for absorption by the casts. In many of our cases known intravascular hæmolysis has occurred, and in others it is difficult to exclude this with certainty.

Such casts are apt to give a positive benzidine reaction, which varies in intensity from cast to cast and from case to case ; this, however, is by no means evidence of hæmoglobinuria or myohæmoglobinuria, since some leakage of red cells, which may subsequently undergo autolysis, into the tubules is common enough in these damaged kidneys.

The appearance of pigment in the casts, which marks the next or *second stage of the lesion*, is observable after some forty hours. The pigmented material tends to appear in granular masses and to be a progressive change in the hyaline casts which were observed at an earlier stage (Fig. 11/17). These casts are seen especially in the second convoluted tubules (Fig. 11/23). They are found in varying, but often very large, numbers all through the lower reaches of the nephron, right down to the ducts of Bellini. At first they appear mainly as amorphous deposits or large rounded granules, rather resembling red corpuscles, but as time goes on they are seen to be more and more contributed to by desquamated necrotic cells which absorb pigment and may be seen in all stages of dissolution, gradually losing their nuclei and being converted into blocks of pigmented material which become agglomerated (Fig. 11/24). At times an appearance of a detached hollow cylinder lining the tubule is seen. As time goes on the most bizarre appearances are often produced, with casts overgrown by masses of recently proliferated epithelium (Fig. 11/25). At the early stage of simple pigmented-cast deposition evidences of necrosis, catarrh and epithelial regeneration, though not lacking, do not especially dominate the picture. Sooner or later, proliferative changes involving renal cells, inflammatory infiltrations, polymorphonuclear activity and interstitial tissue increase begin to dominate the picture. This may be called the *third stage* of inflammatory reaction which becomes first evident at about the fourth to fifth day of the disease and is well developed by the sixth. This stage represents the full development of the histological lesion. The picture was described in detail (Bywaters and Dible, 1942), and has since been analysed on the basis of a very large series of cases (Lucké, 1946), so that it is unnecessary to describe it here. An attempt will be made, however, to find a reason for the development of these structural changes and to correlate them as far as possible with the functional findings. Clinical evidence points to a condition of anuria preceding anatomical renal damage. There is evidence that filtrate is produced—we have referred to

the distended condition seen in the first convoluted tubules in some cases—and in experimental animals there is the well-known observation of Richards (1929) on the production of filtrate in frogs' kidneys rendered anuric by poisoning with mercury perchloride. Lastly, there is the suggestion that the main brunt of the lesion falls upon the intermediate or boundary zone of the organ, and it is proposed to discuss this evidence more fully.

Genesis of the Boundary Zone Lesions. The early necrosis of the descending limb of Henle, mentioned as the first visible change, is followed by a concentration of pathological alterations in this area ; many of the collecting tubules become filled with cast material, the vasa recta are congested and some œdema appears, especially about the descending leashes of these vessels. There is œdema of the papilla and often a ragged desquamation of the cells in the larger collecting tubules and excretory ducts. Certain of the tubules, especially smaller collecting tubules, are beginning to break up. These changes give the suggestion that a veritable pathological barrier is formed across the base of each renal pyramid at this level. In the medulla itself the familiar appearance of blockage of the collecting tubules is much in evidence, with gross casts within them (Fig. 11/26), which may or may not be pigmented according to the duration of the disease.

With the onset of inflammatory changes, at about the fifth or sixth day, these appearances are intensified and the conditions in the boundary zone of the kidney become chaotic. The tubules are in part breaking down and losing their form and individuality in the mass of young cells which is appearing, their epithelium showing both necrotic changes and regeneration. The œdematous tissue between the tubules is becoming increasingly cellular and fibrous in appearance (Fig. 11/27) ; fibroblasts can be seen and many scattered mononuclear cells, as well as focal accumulations of these become prominent (see also Figs. 11/28B and 11/29B). In addition there are vascular lesions, described as " tubulo-venous " by Dunn and his co-workers (1941), which appear to be associated with a focus of inflammatory reaction about a necrotic tubule which impinges upon a vessel, especially the larger veins, and produces a lesion in the vessel wall and a local focus of inflammatory change with or without thrombus formation. Occasionally these lesions may attain a considerable size (Fig. 11/30). In many tubules areas of frank dissolution can be observed, sometimes with an apparent extrusion of cast-like protein material into the interstitial tissue ; these areas are often the seat of particularly active cellular infiltration. Darmady (1950, and personal communications), investigating the nephrons by the corrosion and microdissection technique (Huber, 1911) adduces evidence that these lesions are tubular ruptures with protein effusion, which he distinguishes as " disruptive " (Fig. 11/31), but he states that there are also " adhesive " lesions in which there is no break in the tubule but an

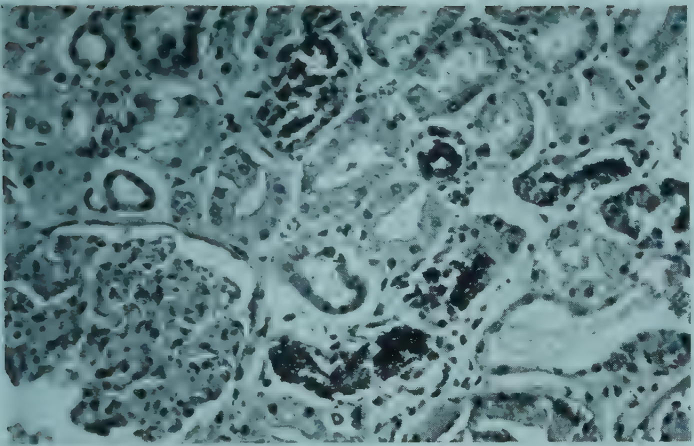
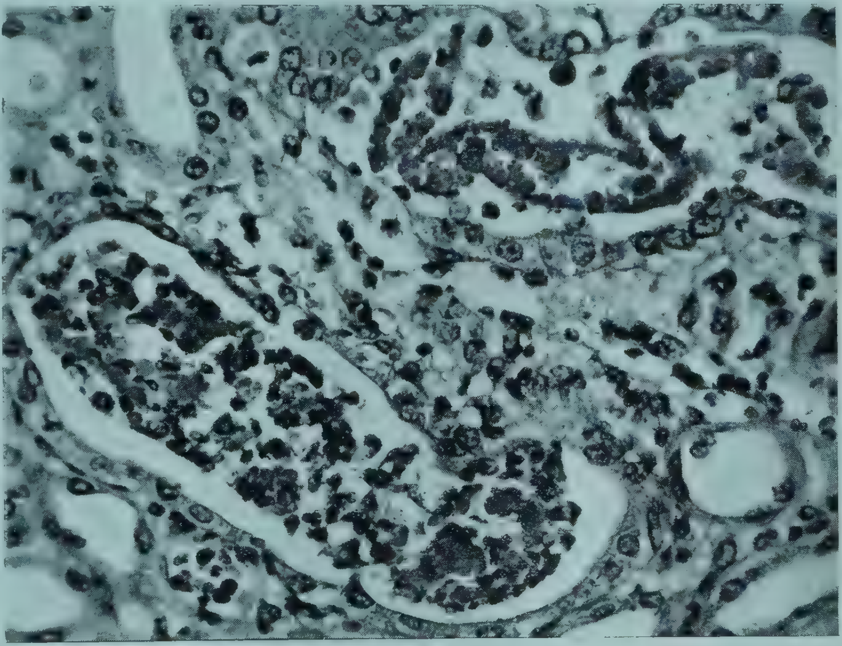


Fig. 11/23. Granular pigmented detritus in the second convoluted tubules. Same case as previous [Figure 11/22] × 130.

Fig. 11/24. Collecting tubules, one showing a cylinder of pigmented material and another desquamated necrotic cells becoming amorphous and pigmented, with some polymorphonuclear invasion. Case of crush: seven days' anuria. × 290.



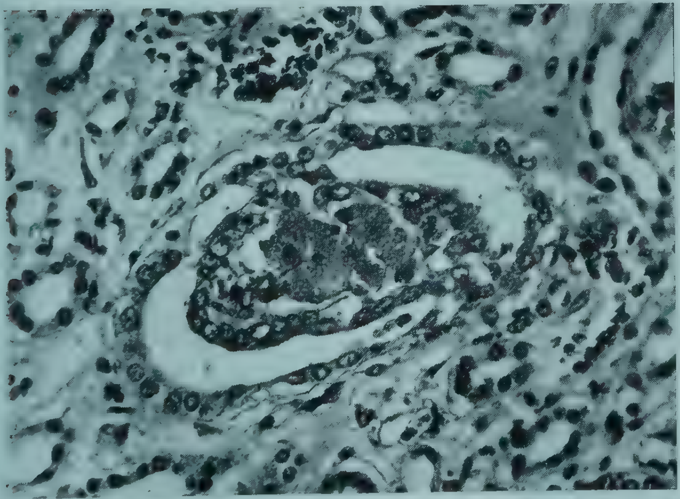


Fig. 11/25. Collecting tubule containing a pigmented cast enveloped by a layer of proliferated epithelium. Case of crush: six days' anuria. × 205.

(Figs. 11/23–11/25 are reproduced from Bywaters and Dible, *J. Path. Bact.*, 1942, **54**, 111, by kind permission.)

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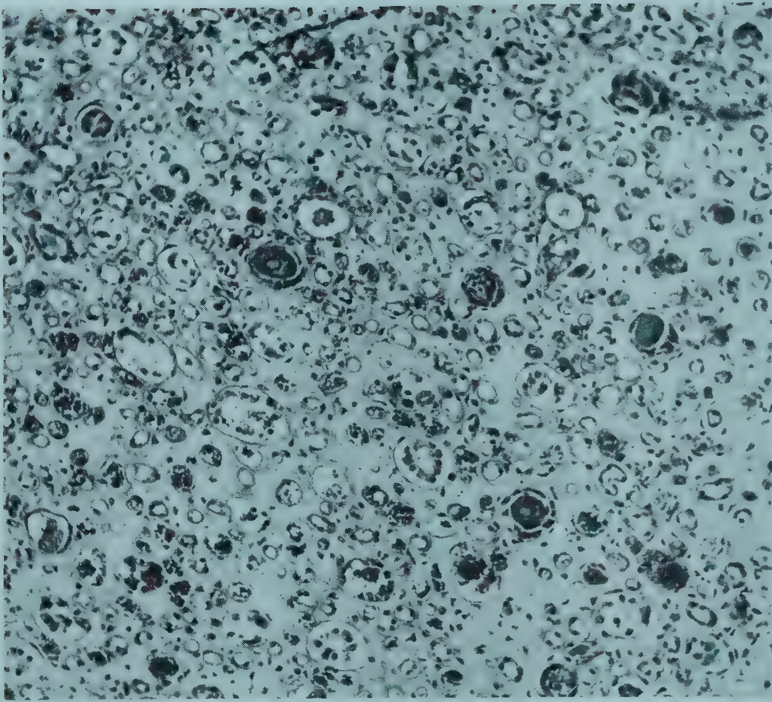

FIG. 11/26. Upper part of medulla showing œdema, cellular degeneration and desquamation, with casts in the collecting tubules. Case of four days' anuria after the operation of œsophago-gastrectomy. × 80.

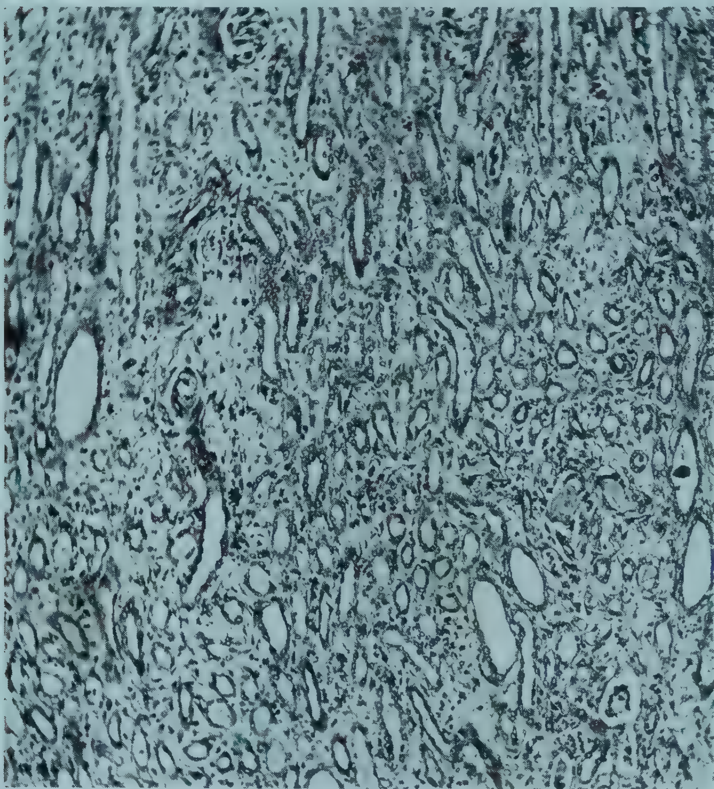

FIG. 11/27. Boundary zone in the kidney of a case of anuria of six days' duration (Rhesus incompatibility).

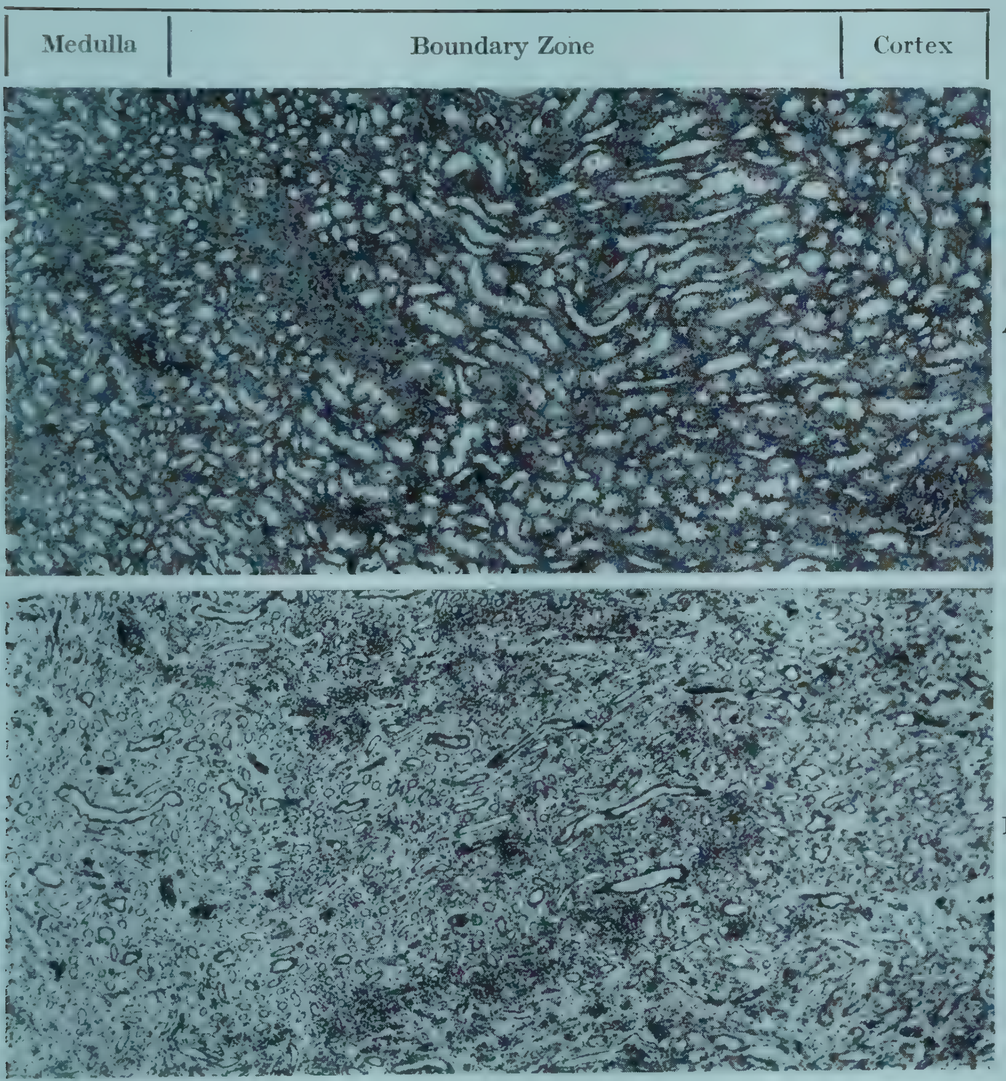

Fig. 11/28. Boundary zones of (A) normal and (B) anuric kidneys, showing degenerate and atrophic tubules, œdema and fibrosis of the intercellular tissue and widespread cellular infiltration. From the same case as Fig. 11/32. Low power view × 36.

A

B

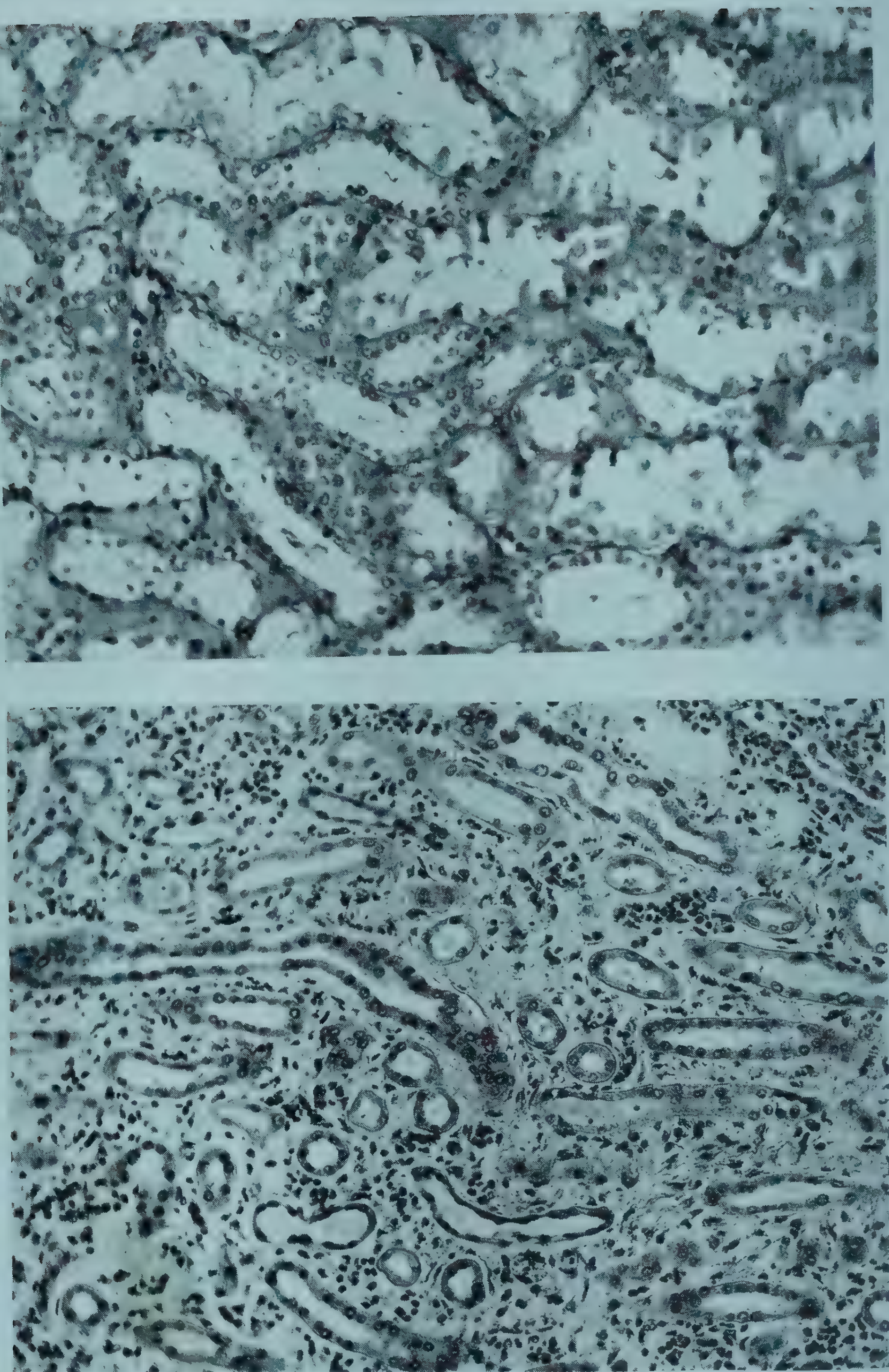

FIG. 11/29. High power view of fields seen in preceding figure. × 160.

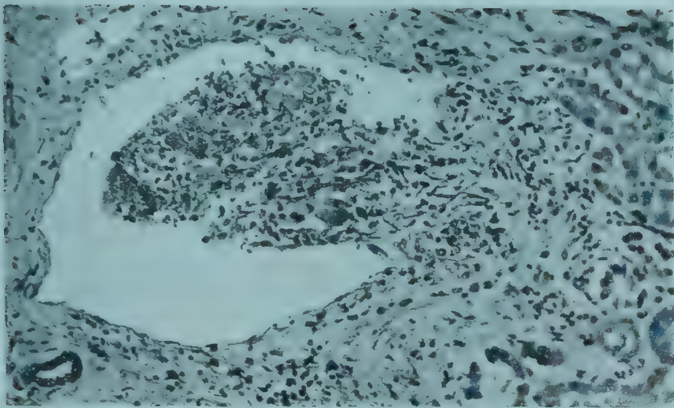

FIG. 11/30. Inflammatory focus in the wall of an interlobular vein with thrombus formation and commencing organization. Crush case in recovery: death from pneumonia on twelfth day. × 97.

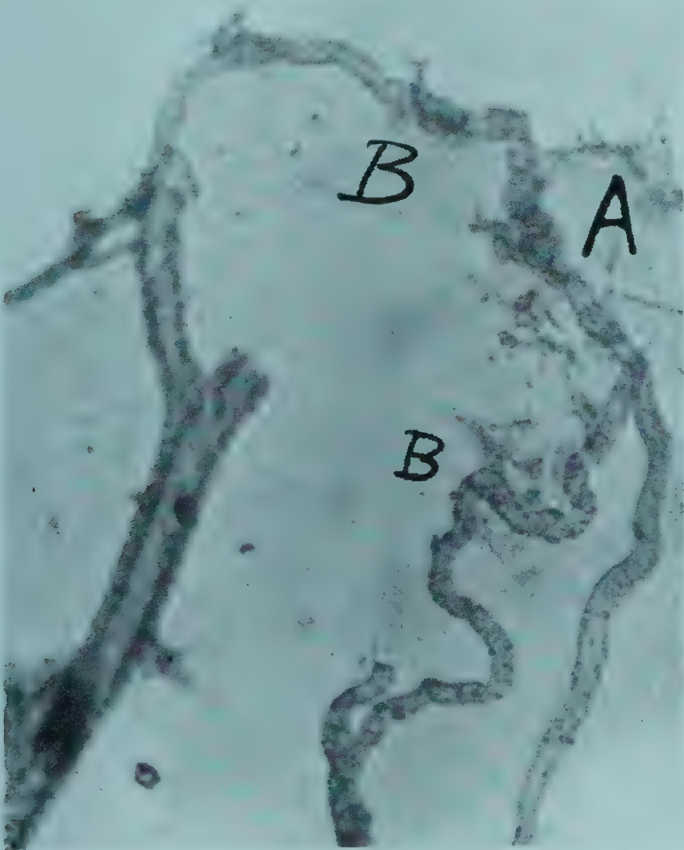

FIG. 11/31. Micro-dissection of the second convoluted and collecting tubules in a single nephron showing A. "Disruptive" lesion; B. "Adhesive" lesions. Note also a pigmented cast in the collecting tubule. From a case of crush. (*Photograph kindly supplied by Dr. E. M. Darmady.*)

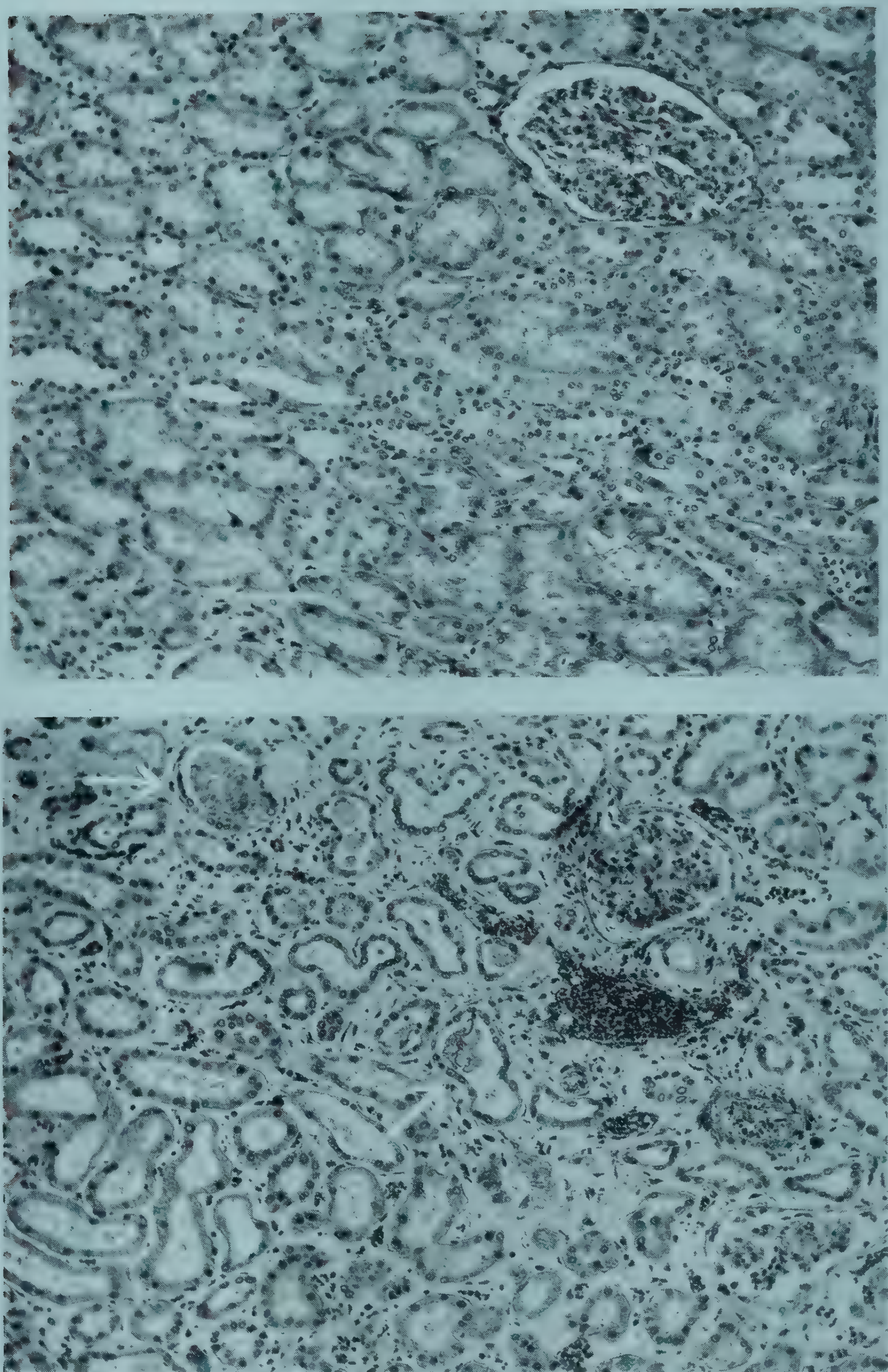

FIG. 11/32. Cortex of normal (above) and anuric (below) kidneys from a case of ten days' anuria (Figs. 11/28 and 11/29 are also from this case).

The cortex of the diseased kidney shows œdematous separation of tubules, the presence of casts in many of the second convoluted tubules, which are concentrated round about the glomerulus, and some alteration in the epithelium of all tubules. × 100.

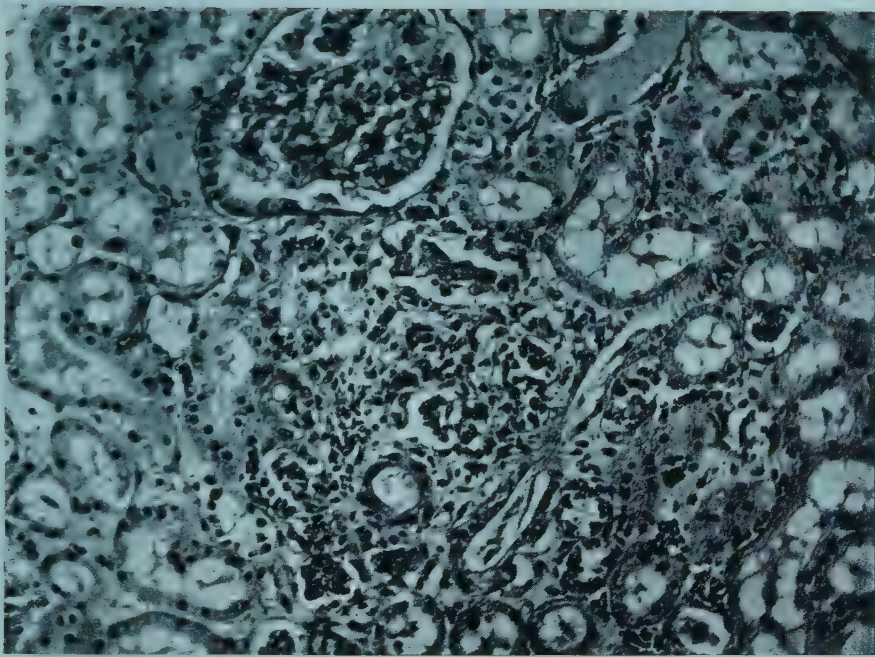

FIG. 11/33. Inflammatory changes and necrosis in the cells of the second convoluted tubules. Crush case of four days' duration. × 145. (Bywaters and Dible, *J. Path. Bact.*, 1942, **54**, 111.)

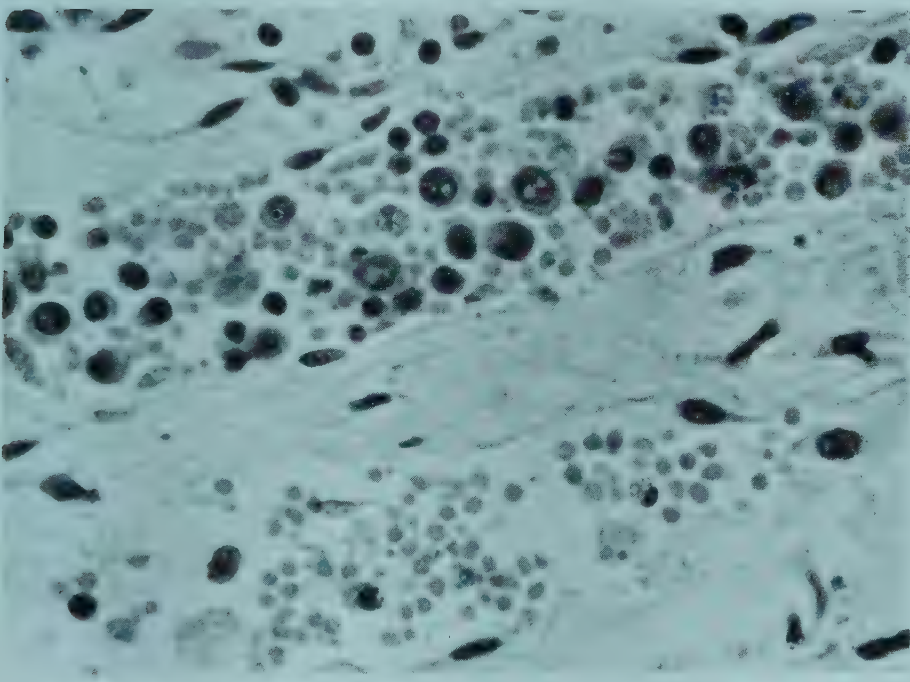

FIG. 11/34. Intravascular cellular accumulations (? hæmopoiesis) in venules of the boundary zone. Case of post-operation anuria of seven and a half days, following peritonitis, operation, "shock" and transfusion. × 550.

adhesion of protein material to its outside. The latter may well correspond to the areas of focal inflammatory change (Fig. 11/28B) just mentioned. Although such lesions are not limited to the lower part of the nephrons they are most marked in them.

It is difficult to appreciate the extent to which the lesions just described, microscopic and often scattered and differing in degree from case to case, affect the whole structure of the kidney but a comparison of the microphotographs of two kidneys from a single subject (Figs. 11/28, 11/29, and 11/32) is of assistance. This material was obtained from a patient who had a nephrectomy performed for hypernephroma and unfortunately at the same time received an incompatible blood transfusion and developed anuria. He died ten days later from pulmonary œdema at a time when clinical observations suggested that the renal lesion was improving. Healthy portions of the kidney removed at operation serve as a control to the anuric kidney. In Fig. 11/32 the cortices are shown. In the diseased organ the obvious changes in this area are the separation of the tubules by cortical œdema, with an apparent increase in fibrous interstitial tissue. Pigmented casts are present in many of the second convoluted tubules; these are dilated, their epithelium flattened and the distinction between first and second convoluted tubules is in part lost.

In the Figs. 11/28 and 11/29, illustrating the intermediate zone, the changes are much more marked. Under low magnification the number of tubules appears to be seriously reduced. This appearance is really due to widespread tubular collapse, in some cases leading to the production of a lumenless column of cells, in others to tubules of minute dimensions. Some of these tubules contain casts. Along with this there is a great separation of the tubules by an œdematous interstitial tissue, heavily infiltrated with inflammatory cells—lymphocytes, plasma cells and large mononuclear cells. The appearances suggest that as far as secretion is concerned these tubules are functionless and they contrast with the rather dilated first convoluted tubules seen in the cortex. The question of whether an actual fibrosis develops in the interstitial tissue at this stage is a difficult one. A mere separation of collagen and reticulin fibres by œdema and cellular infiltration might produce a similar picture. Nevertheless, there does appear to be an actual increase in both reticulin and collagen whilst the presence of numerous fibroblasts is indubitable. We have not illustrated the medulla in this case but it showed the familiar filling of the collecting tubules with casts—so common and striking a picture in most of these cases. In many other cases the collecting tubules are filled with convoluted strands of proliferated epithelium which indicates both catarrh and regeneration and stagnation in these reaches of the nephron. The condition of the second convoluted tubules in the cortex is readily understandable, since they conform to the lesions we have just described, the second convoluted being in fact a cortical extension of the ascending tube of Henle. Hence, in the inflammatory stage of the lesion, we meet the same degenerative changes in these tubules and similar interstitial cellular reactions (Fig. 11/33). Sometimes the necrotic changes in these tubules are associated with an acute polymorphonuclear reaction, so marked as to suggest a bacterial infection, but no bacteria are found.

At a late stage there may be actual and extensive loss of these tubules.

To sum up, therefore, the findings taken together would seem to indicate a relative functional bar to the passage of filtrate, situated in or about the base of the renal pyramid. There is less evidence about the excretory function of the cortex, but such as there is suggests that this is by no means at a standstill.

One other fact must be considered. What are the circulatory conditions in the organ and how do they fit in with the epithelial and other changes ? It seems certain that the circulation through the medulla and juxta-medullary cortex must be physically impeded by the anatomical changes, such as are shown in Figs. 11/28 and 11/29. The glomeruli show no constant changes, after their short initial period of congestion. The vasa recta, on the other hand, are dilated at the time œdema of the boundary zone is developing. Later, and we have noted this from the fifth day onwards, there is a very striking and sometimes extreme accumulation of mononuclear cells in the venules of the medulla and intermediate zone, so the appearance resembles that seen in vessels in leukæmia (Fig. 11/34). This is a local phenomenon, not part of a general leucocyte response, and absent from the cortex. The cells are of various types ; some are plasma cells, others monocytes, others resemble cells of the hæmopoietic series—in fact, cells which are not usually found in the circulating blood. Whether they are formed by intravascular proliferation, which appears likely, or whether they have immigrated from the interstitial tissue is not clear. But whichever may be their origin, their accumulation in these vessels exclusively seems evidence of an extremely stagnant circulation. In fact, the whole zone of cellular accumulation is suggestive of an abnormal proliferation of cells which are able to survive, but lack their normal circulation and physiological connections. This appearance is most common and marked in cases of six days' duration, or longer, and has been found persisting in those having the longest survival—up to eighteen days in our series.

Recovery. Recovery from the more severe grades of this condition would appear to be slow. In one case (from Dr. C. V. Harrison) dying from post-partum hæmorrhage, fifteen days after a severe transfusion reaction associated with jaundice and oliguria, the total daily urinary output being about 2 oz. for a period of seven days, there were active cortical lesions with loss of groups of second convoluted tubules and focal lymphocytic infiltrations

at these sites. The cortical tubules were generally dilated. There was also considerable fibrosis in the boundary zone, rather in advance of that shown in Fig. 11/27. In this zone also many tubules were disorganized and casts present, some of which were calcified. The collecting tubules showed desquamative changes and many still contained pigmented casts. In a second case, in which a renal biopsy was obtained five and a half weeks after post-partum anuria, when tubular functional tests were restored to normal, there was a general restoration of the whole cortex, except for scattered and patchy destruction of some second convoluted tubule areas in the cortex, with adhesions in the related glomeruli—a condition which gave rise to some fine scattered scars.

Relationship to Cortical Necrosis. It does not appear that the lesion described is in any way a stage in the development of symmetrical cortical necrosis. From the histological standpoint the latter is essentially a vascular lesion and its affinities lie rather with acute glomerulo-necrosis (Dunn and Montgomery, 1941) than with tubular necrosis. In reviewing this series we have encountered three cases which fall into the group of glomerulo-necrosis. All show changes which are outstandingly glomerular—in pronounced contrast to our cases of tubular necroses. Moreover, the kidneys were generally very hyperæmic and many of the glomeruli showed pronounced vascular stasis, going on to hæmorrhagic necrosis, akin to that seen in the less affected glomeruli in symmetrical cortical necrosis. It is true that there are, as is to be expected, associated tubular changes, but the prominence of the glomerular change justifies the inclusion of these cases in a separate and distinct group. Ætiologically, two of our three cases were cryptogenic and diagnosed clinically as acute nephritis ; the third was a case of concealed accidental hæmorrhage and pregnancy toxæmia.

The Hepato-renal Syndrome

This rather nebulous entity, which appears and disappears from the pathological arena as opinion varies about hepatic and renal pathology, is worthy of consideration. It is true enough that under certain conditions death in jaundice with necrosis in both liver and kidneys may be seen. It seems to us that cases which may claim inclusion in this class are those in which there is a high concentration of bilirubin in the plasma and, along with this, the existence of such factors as promote renal tubular necrosis. When these conditions combine, they produce the

histological picture of renal tubular necrosis with the necrotic tissues stained with bile. It seems likely that the bile pigment is inimical to cell life and aids and abets anything else which tends to kill the cells ; thereafter it stains them. At the same time the picture in the liver, of whatever the condition may be which is responsible for the high serum bilirubin, will be plain to see, with the added factor that the circulating bile may aggravate the liver-cell necrosis, especially in moribund cells. This autolysis may progress considerably after death. The effect of bile in this respect, and its affinity for staining moribund liver cells has been commented upon elsewhere (Dible, 1951). Cases in which we have observed the combination of lesions just described have been for the most part cases of obstructive jaundice which have been subjected to severe operations, such as pancreatectomy. The kidneys are apt to show severe necrosis, especially of the first convoluted tubules, with the collecting tubules full of pigmented casts and desquamated necrotic cells ; the picture is not unlike the one we have described of selective tubular necrosis, but the inflammatory element is absent and the casts do not give a benzidine reaction unless there is associated hæmolysis.

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THE PATHOGENESIS OF ACUTE TUBULAR NECROSIS

Our present view of the pathogenesis of acute tubular necrosis may be briefly tabulated as follows :

Toxins | Ischæmia

Tubule cell damage + vessel wall damage

Leakage of tubule content into interstitial tissue

Rise in intra-renal tension

Mechanical obstruction to renal blood-flow

Reduction in glomerular filtration

Regeneration of tubules

Fall in intra-renal tension

Rise in renal blood-flow

Differentiation of tubule cells

Almost full functional recovery.

blood conglutination in vessels

Cortical necrosis

The ætiological background is usually complex, a toxic factor operating in the presence of renal ischæmia. This combination of factors is also found in experimental tubular lesions when it is frequently impossible to produce tubular necrosis by means of a nephro-toxic substance unless the animal is shocked. This is certainly true in the case of the tubular lesions of experimental hæmoglobinuria (Yuile *et al.*, 1945). Tubule damage is invariable and the whole tubule system shows functional incompetence, but the histologically visible damage is greater in some parts than in others. Massive tubular necrosis appears to be commoner in the toxic varieties, while selective lesions predominate in the ischæmic and mixed types. In patients dying early, at a period corresponding to the onset phase and early part of the anuric or oliguric phase, the tubules may appear almost normal with ordinary stains, but specific staining reveals damage to enzyme systems. The leakage of fluid into the interstitial tissue can be demonstrated histologically and its presence can be inferred from the increased kidney weight. There is no completely satisfactory evidence of a rise in intrarenal tension, but where decapsulation has been performed in life it has been reported that the kidney is tense and bulges out of the cut capsule. The reduction in renal blood-flow is shown by functional tests and in the oliguric phase is extreme. Histological evidence of extremely slow circulation is shown on p. 300. This reduction in renal blood-flow is probably produced mechanically by the rise in intrarenal tension. It is not due to neural factors in established anuria, for sympathetic denervation of the kidney does not alter the course of the illness (Bull *et al.*, 1950). Humorally induced reduction in renal blood-flow is also unlikely, for there is no evidence in other parts of the circulation of the effects of excessive quantities of circulating vaso-active materials (Bull *et al.*, 1950). The glomerular filtration rate must be very low for filtration is dependent on blood-flow. Some glomerular filtrate must be formed, however, for the intra-tubular tension must be higher than in the surrounding tissue, otherwise the tubules would be collapsed. Regeneration of tubules can be seen histologically, but full recovery of tubule function is delayed for some time after diuresis has commenced. Although no histological counterpart of the early diuretic phase can be recognized by ordinary staining methods, cell dysfunction may be visible when enzyme stains are used.

In the acute tubular necrosis associated with concealed accidental hæmorrhage and in experimental toxic tubular necrosis

(Duguid, 1936) numbers of nephrons may be destroyed. This is probably invariable in any severe example of the condition. Cortical necrosis is dependent upon severe vascular damage. The fact that the renal blood-flow seldom returns to normal is probably explained by the loss of nephrons, and when recurrent attacks of acute tubular necrosis occur a contracted kidney may result.

A number of patients die as a result of the primary illness, but when these are excluded death is due to "uræmia" resulting from the deficiency of renal function. "Uræmia" has been defined as the clinical state that results when the kidney is unable to maintain a normal *milieu intérieur*. The word "uræmia" is unfortunate, for it suggests intoxication with urea or urine and a single easily recognized state. This is not the case. There are many "uræmias," and many of them result from disturbances in body content of such simple compounds as water and salts, and may be in the direction of excess due to urinary retention or deficiency due to uncontrolled urinary losses. In the "uræmias" of chronic renal disease the position is so complex that it is usually impossible to decide what factors play the major *rôle*, but in acute uræmia such as occurs in acute tubular necrosis it is frequently possible to decide the particular disturbance which is responsible for the symptoms and signs. In acute uræmia alterations in body content of three groups of substances are responsible for most of the manifestations—water, minerals and end-products of nitrogen metabolism (Bull *et al.*, 1949). A summary of the more important disturbances is as follows :

1. A combined excess of water and sodium causing œdema, cardiac failure and death in pulmonary œdema.
2. A simple water excess causing water intoxication with mental disturbances, stupor, coma, convulsions and death.
3. Water, or water and salt, loss or sodium excess without water excess causing cellular dehydration with stupor, twitchings, coma and death.
4. Potassium excess causing death due to cardiac arrest.
5. Potassium deficiency causing paralysis similar to family periodic paralysis.
6. Acidæmia, with characteristic breathing, stupor and coma.
7. Tetany due to lowering of serum calcium secondary to phosphate retention.
8. Enteritis, colitis, gastritis and stomatitis, probably due

to urea being split by organisms at these sites to form ammonia with consequent mucosal damage.

In addition to these manifestations secondary infection is common, particularly infection of the urinary tract consequent on the low urine flow. The clinical picture and the causes of death in the anuric or oliguric phase are usually the result of accumulation of substances, while in the early diuretic phase are usually due to uncontrolled urinary losses in excess of intake.

Principles of Treatment

Recovery in acute tubular necrosis is almost certainly dependent on regeneration of the damaged tubular epithelium, a process which may take several weeks. Provided the patients can be kept alive during this time, there is no reason why the vast majority should not recover. Treatment under the following headings should be aimed at prevention of those uræmic manifestations which may prove fatal.

Prophylaxis. This includes an attempt to avoid or reduce renal ischæmia by vigorous treatment of the disturbance in the general circulation, and the use of specific antidotes to poisons or preventing their absorption by appropriate measures.

In the onset phase of the toxic acute tubular necroses and those in which both toxic and ischæmic factors play a part, measures directed towards maintaining a dilute urine in the tubules may prevent the damaging high concentration of the poison. This can sometimes be achieved by administering 1 to 2 litres of water or an osmotic diuretic at this time, but it must be remembered that if diuresis fails to occur there is no renal route for loss of the water or diuretic. In the case of water this is not of great importance, for normal water balance can be re-established within twenty-four to forty-eight hours by omitting further water intake during the next one to two days. The excess of water is then lost through the lungs, skin and in fæces. There is no similar way for removing osmotic diuretics, such as sodium sulphate, from the body, so these are best avoided. Glucose acts as an osmotic diuretic when the plasma concentration is high, and has the advantage that it is metabolized to water and CO_2, which can be lost by routes other than the kidney. The rapid administration of 1 to 2 litres of 20 per cent. glucose intravenously therefore is the safest means of achieving the desired result. Once necrosis has developed no measures of this type can be effective.

Correction of imbalances, once they have been developed, is a possible method of treatment. This involves, in most instances, the use of one of the various dialysis methods, the artificial kidney, peritoneal or intestinal dialysis. All dialysis methods are difficult to control and are subject to many complicating factors which seriously limit their usefulness. Some correction of disturbances is usually possible in the deficiency type of disturbance by appropriate feeding, and in the case of potassium retention it is possible to remove potassium from the body by feeding suitable ion exchange resins. On the whole, this method of treatment, which allows disturbances to develop and then attempts correction, is less satisfactory than the prevention of imbalances by careful adjustment of the dietary intake so as to maintain a normal balance. This involves a control of the intake of water, minerals and protein. To maintain a normal water balance in an anuric patient approximately 1 litre of fluid should be given per day, as this balances the insensible losses. When urine is passed, fluid of an equal volume must be added to this basic litre. The anuric patient has no means of excreting appreciable quantities of any mineral so his diet should be completely mineral-free. In particular, it is important that no potassium be given. When urine is passed, and particularly in the early diuretic phase, large quantities of sodium, chloride and potassium may be lost without regard to the body's needs. This loss must be exactly compensated by feeding an equal quantity of these ions. In the case of sodium and chloride the extent of the loss can be predicted with a sufficient degree of accuracy without chemical analysis of the urine, for the concentration of these ions is approximately half that in plasma during the oliguric and early diuretic phases (see p. 288). In the case of potassium no simple method exists for predicting urinary loss, but in practice it has been found sufficient to feed a diet high in fruit during the early diuretic phase and watch for signs of potassium deficiency, which can be corrected by further potassium administration as required. Dangerous degrees of potassium excess may develop during the anuric or oliguric phase if potassium containing foods are fed and also may arise from release of potassium from the patient's own tissues. The exogenous sources of potassium are easily eliminated, and the tissue release of potassium can be considerably reduced in the following way : If large quantities of carbohydrate and fat (2,000 or more calories per day) are fed, the rate of endogenous protein breakdown and parallel potassium release is

markedly slowed. Indeed, the serum potassium may even fall in anuric patients on this type of diet as potassium forms a harmless compound with phosphate and glycogen when the glucose is stored. During the anuric phase no protein should be fed, for this will be converted to urea, and furthermore most protein-containing diets also include appreciable quantities of potassium.

The needs of the anuric subject are therefore 1 litre of fluid per day, no protein, no minerals and 2,000 or more calories in the form of carbohydrate and fat per day. When urine is passed during the early diuretic phase exact balance of intake and urinary output of water and minerals is necessary. During the late diuretic phase the kidney has regained its ability to select or reject the substances required by the body and no further treatment is necessary. The early diuretic phase lasts on the average for as long as the period of anuria or oliguria which precedes it, and it is therefore possible to predict when control of intake may be relaxed. A diet such as is indicated in the anuric phase is unpalatable and nauseating. In practice a synthetic form, fed through an in-dwelling gastric tube, has been found to have many advantages (Bull *et al.*, 1949). By this means a constant and steady intake can be estimated during the twenty-four-hour period, and it is possible to return vomitus to the stomach, so avoiding unknown loss of fluid minerals and food. In addition to the dietary treatment, penicillin should be administered as a prophylactic measure against infection, since the latter encourages protein catabolism and lessens the protein-sparing effect of the diet. If the hæmoglobin falls below 70 per cent. (Haldane), packed red cells should be transfused for anæmia lowers the rate of renal blood-flow.

Several measures have been found to be contra-indicated or proved ineffective. These include :

i. The administration of diuretics other than water and glucose in the onset phase. No diuretic can be effective when glomerular filtrate is not being formed. Mercurial diuretics in particular are contra-indicated, for they will cause further tubular damage.

ii. There is no good evidence that sympathetic block or spinal anæsthesia is effective in established anuria (Bull *et al.*, 1950), and the diminished renal blood-flow is probably caused by local mechanical factors in the kidney.

iii. Although there is evidence to suggest that the cause of the reduced renal blood-flow is a rise in intrarenal tension, decapsulation is ineffective (Culpepper and Findley, 1947). This is probably because it is the rigid stroma of the kidney, particularly at the juxtamedullary region, and not the capsule which determines the degree of pressure rise.

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CHAPTER XII

THE DUCTLESS GLANDS

Endocrinology

THE endocrine function of a gland is proved when the results of its removal have been described and have been remedied by administration of a hormone derived from the gland. The proof is clinched when over-dosage of the hormone reproduces the symptoms of pathological over-activity of the gland.

Endocrinology has been developed by anatomists, pathologists, physiologists and biochemists—roughly in that order. The first described the glands without ducts and with no known use; the second related diseases to pathological changes in these glands; the third proved that their function was to make hormones and secrete them into the blood; the last revealed the chemical nature of these hormones.

Most of the hormones have now been isolated and purified. The chemistry of the steroid hormones of the gonads and adrenal cortex was mainly unravelled during the 'thirties and the main protein hormones of the hypophysis were separated and purified during the 'forties. The chemistry of the hormones is now more a matter for the pure chemist, while the biochemists turn their attention to the mechanisms of hormone synthesis and action in the body.

Endocrine Diagnosis. The thyroid hormone and insulin were the first two hormones to be used clinically and their success was dramatically obvious. On the whole, the benefits derived from the use of the sex, adrenal, and hypophysial hormones have not been quite so striking. This is partly because the disorders they have to correct are more complex and rigid diagnosis is therefore more difficult. Insulin and thyroxine produce effects on the blood-sugar level and on basal metabolism that are fairly simple to measure: a useful aid in diagnosis, investigation and treatment. The effects of the sex, adrenal and hypophysial hormones are usually not so easily measured, at least by methods that can be used on patients. Hence the importance of devising tests that can be applied to assess the functional capacity of the various endocrine glands and of discovering direct or remote effects of the hormones that can be easily measured to the same

end. Other factors hindering accurate diagnosis are the interlocking relations between the different endocrine glands and the predominant *rôle* of the anterior pituitary (adeno-hypophysis). The under- or over-activity of a gland may have its origin in a disorder of some other gland, and the distinction between primary and secondary disorders is often difficult.

Hormone Elimination. The best way to assess the functioning of an endocrine gland would be to measure its output of hormone. The amount of hormone secreted into the blood is quite large, but the amount in a single sample of blood is very small, too small to allow its accurate measurement at present. For this reason an indirect method of measuring hormone secretion has developed depending on the estimation of the hormone or of its metabolites in the urine. Such estimations can be made on a larger bulk of material and with simpler chemical procedures than are possible with blood. For the steroid hormones there are routine chemical procedures that will measure the urinary products with accuracy, but biological assays still have to be used for the protein hormones and these are slow and not usually very accurate.

Even though the steroid products in the urine can be measured accurately, the results are not necessarily an accurate index of the rate of hormone secretion ; once the hormone is secreted into the blood there are still many factors that may influence the amount and nature of the products that will appear in the urine.

Endocrine Treatment. Once a disorder has been traced to an alteration in the function of an endocrine gland, treatment must either make good the deficiency of hormone or revoke its excess. Hyperplasia or over-activity of a gland is treated by surgical removal of all or part of the gland. Thyroid over-activity can also be treated by giving anti-thyroid drugs that prevent the formation of thyroid hormone, but such pharmacological treatment of over-activity of other glands is not yet possible.

Deficiency of hormone can be remedied by giving the missing hormone—so long as the hormone is administered, normal conditions can be maintained, as in myxœdema and in diabetes insipidus and mellitus. Usually, however, such a procedure effects no cure. It is only when there is a failure of some cyclic function regulated by the mutual relations of two hormones, such as in the regulations of the ovarian cycle, that hormone

treatment may effect a cure. The cycle may have stopped through a temporary failure of one of the hormones, and if it can be re-started artificially it may continue of its own impetus.

Hormone Administration. Dried thyroid is fully effective when given by mouth and artificially prepared derivatives of some of the steroid hormones can also be given in this way; otherwise the natural hormones have to be given parenterally. An efficient means of doing this with the steroid hormones is by implanting tablets subcutaneously; their low solubility in body fluids means that slow absorption continues over weeks or months, making this the method of choice where prolonged action is required with the minimum of hormone wastage and inconvenience to the patient. Synthetic analogues of the œstrogens have been prepared, but not, so far, of other hormones. These compounds, of which stilbœstrol is the most widely used, are products of the chemical laboratory that have all the properties of the natural œstrogens, and are in addition active by mouth, while being chemically much simpler and cheaper to prepare than the natural hormone.

Antihormones. An interference complicating treatment with protein hormones of the adenohypophysis or placenta is the formation of antihormones in the blood. These antihormones neutralize the normal effects of the hormones, so that treatment eventually, usually within a matter of weeks, becomes useless. Some believe that antihormones are natural, physiological things but this is certainly not proved. Most of the work on antihormones has been carried out with impure hormones generally prepared from a species other than that of the injected animal or patient. Naturally the hormones used clinically will always be derived from non-human sources so the species difference will remain, but perhaps when the hormones are available as pure proteins they will no longer be antigenic. Insulin is not antigenic, even if impure preparations are administered and the possible antigenicity of the antidiuretic hormone is not enough to interfere with continued treatment.

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THE ADENOHYPOPHYSIS

Introduction. The adenohypophysis consists of the partes distalis, intermedia and tuberalis. The pars intermedia which controls melanophore expansion in fishes, amphibians and reptiles, is poorly represented in man where it has no known function. The pars tuberalis is as clearly distinguishable in man as in lower animals but has no certain function in either. Known endocrine function of the adenohypophysis in man is confined to the pars distalis. Evidence for the secretion of hormones controlling the thyroid, adrenals and gonads will be discussed later. In addition to these a protein hormone directly influencing growth (growth hormone or somatotropin) has been isolated from the pars distalis and purified. At least six hormones are thus secreted by a gland which is composed of only three main types of cell : chromophobe, acidophil and basophil cells. There is no cytological or physiological evidence that the chromophobe cells are secretory, and they are usually regarded as transitional non-secretory forms representing immature or exhaustion phases of the secretory cells. Since the basophil cells increase in number after castration in animals in parallel with an increase in gonadotropic potency and are increased in post-menopausal women who excrete increased quantities of urinary gonadotropin, these cells have been regarded as the source of gonadotropin. If this is so then it must be the follicle-stimulating hormone as there is evidence that the luteinizing hormone is formed in the acidophil cells. These cells become very granular after coitus in the rabbit and then lose their granules before ovulation occurs ; the inference is that these carminophil granules are the luteinizing hormone. Similar increase and release of carminophil granules before parturition and during early lactation in cats suggest that luteotrophin is also formed in these acidophil cells (Dawson, 1946). The origin of so many different protein hormones from two cell-types has suggested that their separation is a chemical artefact and that fewer proteins are actually secreted which are larger molecules and have more than one hormonal activity.

Growth Hormone. Young animals that are hypophysectomized stop growing and old ones lose weight—both effects are overcome by growth hormone. In the young animal the hormone is not only responsible for the increase in weight, but also for growth of the skeleton. Almost normal bone growth will continue so long as the epiphyses are not closed, and in species such as the rat, where such closure is late, giants can be produced by continued treatment. If the epiphyses are already closed when

treatment is started then abnormal bone growth and thickening occur. The effects of growth hormone on body weight are produced by protein anabolism ; during the period of administration there is nitrogen retention and decreased urea formation ; the water content of the body increases and the fat content is reduced.

The clinical effects of under- or over-production of growth hormone are seen in pituitary dwarfs, giants and in acromegalics. The pituitary dwarf usually has a multiple deficiency so that thyroid and gonadal functions are also defective and development is influenced. The conditions of gigantism and acromegaly are usually caused by pituitary tumours and show effects of excess growth hormone combined with gonadal deficiency. The tumours causing acromegaly are usually composed of chromophobe or acidophil cells—evidence that the eosinophil cells are the source of growth hormone. The chromophobe cells are then regarded as degenerate or exhausted chromophil cells and tumours composed of these cells are presumably no longer actively secreting. Pressure on the basophil cells and their partial or complete obliteration will then account for the gonadotropin deficiency. The source of thyrotropin is disputed : increased basal metabolic rate and goitre are often found in acromegalics, suggesting that thyrotropin also comes from the eosinophil cells. Tests of thyroid function in acromegalics with raised basal metabolism have not, however, always indicated any increased thyroid function. The question still remains open. The skeletal changes in gigantism are like those produced in young animals by growth hormone ; in acromegaly the changes resemble those produced in adult animals.

Gonadotropic Functions. The actions of the two gonadotropic hormones that have been isolated from the adenohypophysis have been most extensively studied in the rat. In the intact rat either hormone will produce mixed effects owing to endogenous gonadotropin secretion but in the hypophysectomized rat the effects are clearly distinct. Follicle-stimulating gonadotropin will stimulate follicular growth but will not provoke œstrogen secretion, ovulation, or luteinization. Luteinizing hormone by itself will only produce ovulation if ripe follicles are already present in the ovary—otherwise it has little effect unless given in large doses, when it will cause luteinization of immature follicles without ovulation. A small proportion of luteinizing hormone added to the follicle-stimulating hormone will enhance the action of the latter so that the ripening of the follicles is accelerated and œstrogen secretion is initiated.

These facts have led to the following hypothesis regarding the regulation of the œstrous cycle in the rat. The secretion of follicle-stimulating hormone and a small proportion of luteinizing hormone leads to follicle ripening and œstrogen secretion. As the œstrogen concentration in the body rises it provokes a release of luteinizing hormone and a suppression of the release of follicle-stimulating hormone. The evidence in favour of this hypothesis will necessarily be indirect so long as it is impossible to assay the blood levels of each gonadotropin when the relative proportions of the two are unknown. The clearest evidence that secretion of follicle-stimulating hormone is suppressed by ovarian secretion probably comes from experiments in which rats are spayed and part of one of the removed ovaries is transplanted into the spleen. This procedure leaves a portion of ovary in the body while effectively preventing ovarian œstrogen from entering the circulation since it is destroyed by passage through the liver. (This hepatic inactivation is easily demonstrated by implanting pellets of natural œstrogens into the spleen of spayed animals, when no œstrogenic effects are produced although implantation under the skin or elsewhere outside the portal circulation reveals full œstrogenic activity ; stilbœstrol does not show this difference, and this resistance to hepatic inactivation explains its oral activity.) Under these conditions the uterus and vagina become atrophic as in the spayed animal while the ovarian fragment in the spleen shows overgrowth which may eventually lead to tumour formation. The gonadotropic potency of the pituitary glands is increased in such experiments just as it is after spaying or castration (Li and Gardner, 1949). Since corpora lutea are formed in the intrasplenic grafts the absence of ovarian œstrogen does not prevent the secretion of luteinizing hormone and the evidence that œstrogen stimulates release of this hormone is not so well founded. It may be that the ripening of the follicles or the presence of sufficient œstrogen alters the sensitivity of the follicle to luteinizing action instead of any action on the adenohypophysis being involved.

It is difficult to say how far these experimental findings are relevant to adenohypophysial control of the menstrual cycle, but the evidence suggests that similar relations are concerned. The urinary output of gonadotropin and œstrogen are both maximal at the point in mid-cycle when ovulation occurs and there is a much greater output of urinary gonadotropin after ovariectomy or the menopause, and this gonadotropin is predominantly, but not solely, follicle-stimulating in action.

The adenohypophysial gonadotropins have only been purified on a laboratory scale so that clinical treatment of ovulatory failure has to depend on impure mixtures of animal material or on gonadotropin from pregnant mares or chorionic gonadotropin from the urine of pregnant women. The serum gonadotropin is predominantly follicle-stimulating, while the action of chorionic gonadotropin is very similar to that of luteinizing hormone, and some success has been achieved in inducing ovulation by injecting the former for several days to produce ripening of the follicles before giving an intravenous injection of the latter to ovulate them. As both hormones are antigenic and are being used for purposes other than those they serve in nature it is not surprising that successes with such treatment are exceptional. A further unknown quantity in treatment of ovulatory failure is the status of the ovary—the response of the atrophic ovary to gonadotropic stimuli is very different from that of the normal organ.

Luteotrophin. The existence of a luteotrophic factor had been deduced many years before its identity with prolactin was established. The known gonadotropins could produce ovulation and corpus luteum formation in hypophysectomized rats but no progesterone was secreted ; neither implantation nor deciduomata could be induced in the uterus. These effects can be elicited if prolactin is injected after corpora lutea have been formed under the influence of gonadotropic stimuli. It is probable that prolactin exerts its luteotrophic action during the luteal phase of the menstrual cycle but its action, as also in the rat, is a limited one in time. In the rat injected with prolactin a state of pseudopregnancy is produced but this only lasts for the usual ten to twelve days despite continued injections. Similarly, in monkeys and women menstruation cannot be delayed by injections of prolactin, although the corpus luteum is still responsive to a different luteotrophin—as is shown by the prolongation of its functional existence produced by chorionic gonadotropin. Again this second stimulus will only exert its effect for a limited time and menstruation is only delayed for ten to fifteen days although the injections are continued. Evidently the corpus luteum has an innate tendency to undergo degeneration and this can be postponed but not prevented (White, 1949).

Control of Function. High concentrations of thyroidal, gonadal, or adrenal-cortical hormones usually depress the secretion of their appropriate adenohypophysial regulators while low concentrations increase secretion. Such regulations are for

the most part effected by direct action of the circulating hormone on the adenohypophysial cells. Thus more or less normal endocrine function can be maintained in experimental animals in which the pars distalis has been transplanted into a different site. Such independence of the adenohypophysis from hypothalamic regulation may not be complete. In the rabbit, section of the pituitary stalk prevents the ovulation that normally follows copulation and similarly prevents pseudopregnancy in the mated rat. Section of the stalk in other animals has produced temporary aberrations in the sex cycle and it is quite possible that many of the finer adjustments of endocrine function are mediated in this way. Electrical stimulation of the hypothalamus produces ovulation in rabbits but the means by which the stimulus passes to the adenohypophysis is debated. The scantiness of the nerve connection has suggested that a humoral mechanism is involved and the pituitary portal circulation is invoked as the route. Since ovulation can be produced by adrenaline injections there is clearly an adrenergic link and probably a cholinergic one preceding it (Markee, 1951).

Clinical Deficiency. The symptoms of Simmond's disease are those that would be expected—the gonads, thyroid and adrenal cortex atrophy, sex function fails, basal metabolism is lowered and incipient Addison's disease can be demonstrated when adrenal-cortical function is tested under stress. The condition arises by obliteration of the functioning pars distalis either by chromophobe-cell adenomas, by tumours of neighbouring structures, or by post-partum hæmorrhage and necrosis. Urinary excretion of gonadotropin, 17-ketosteroids, and corticosteroids are greatly reduced. Patients with Simmond's disease, like hypophysectomized animals, may survive for long periods provided they are not exposed to stress or illness. If death is not preceded by intercurrent illness it generally occurs in coma, often precipitated by anorexia and vomiting, factors supporting the obvious inference that adrenal-cortical deficiency is the most dangerous consequence of the adenohypophysial failure (Sheehan and Summers, 1949).

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THE NEUROHYPOPHYSIS

The neurohypophysis consists of the pars nervosa or infundibular process, the infundibulum (infundibular or neural stalk) and the median eminence of the tuber cinereum. Histologically all these divisions of the neurohypophysis have common features and the gradual merging of this tissue into the floor of the hypothalamus makes complete operative removal of the gland extremely difficult. The gland is largely composed of nerve fibres and endings interspersed, particularly in the pars nervosa, with neuroglial cells, sometimes called pituicytes, which are regarded as secretory. Extracts of the gland contain a protein or proteins which have pressor, antidiuretic and oxytocic activities. The non-medullated fibres present throughout the gland and forming the chief component of the stalk arise in the supra-optic, paraventricular, and tuberal nuclei of the hypothalamus. The clearest experimental proof of the normal function of this hypothalamico-neurohypophysial complex is the production of diabetes insipidus in experimental animals by destruction of the nerve tract anywhere along its course. This destruction is followed by atrophy of the hypothalamic nuclei and of the neurohypophysis with loss of its hormone content.

Neurohypophysial Hormones. Although extracts of the gland have three main activities, only two substances have been separated and purified. These are the pressor/antidiuretic factor and the oxytocic factor, both polypeptides of relatively low molecular weight, but a larger protein molecule has also been purified which contains the two activities in constant proportions. At present it cannot be decided whether the larger molecule forms the secretion of the gland and the two smaller ones are laboratory artefacts, or whether the larger molecule is the form in which the hormones are synthesized and stored within the pituicytes, only to be broken down and released as separate smaller hormones when required (Stehle, 1949a). There is only indirect evidence that the pressor and antidiuretic activities of neurohypophysial extracts are distinct, and until a separation is actually accomplished or any physiological *rôle* can be attributed to the pressor activity it can be assumed that this activity is a pharmacological property of the antidiuretic hormone in excess. If this interpretation is correct then the name vasopressin will eventually have to be superseded.

Diabetes Insipidus. There is plenty of evidence that the antidiuretic factor is a hormone and that its absence causes extreme polyuria. Such polyuria soon appears after removal of 90 per cent. of the neurohypophysial tissue in experimental animals and the increase in urine excretion precedes the increase in water intake, showing that polyuria is primary and the polydipsia

secondary. The same polyuria is produced if atrophy of the neurohypophysis is produced by destructive lesions of the supra-optic and paraventricular nuclei in the hypothalamus or of the nerve tracts from these to the infundibular stalk. Perfusion experiments demonstrate that the antidiuretic hormone acts directly on the kidney. It does not appear to alter glomerular filtration rate but increases the tubular reabsorption of water and decreases that of chloride ; thus dehydrated animals given anti-diuretic hormone excrete less water but more chloride than normal during the time that the hormone is acting (Stehle, 1949b). The rate of secretion of the hormone is controlled by the osmotic pressure of the blood ; when this is increased by intracarotid injection of hypertonic solutions in hydrated dogs an antidiuresis results. The osmoreceptors are situated somewhere within the vascular supply of the internal carotid artery (Verney, 1947). There is also an increased urinary excretion of the hormone during dehydration. The detection of antidiuretic activity in urine and blood is still experimental, but it is fairly successful in urine. Injection experiments have shown that the hormone is destroyed or inactivated in the liver and this probably accounts for its high rate of urinary excretion in cases of hepatic cirrhosis (Ellis and Grollman, 1949). The full symptoms of diabetes insipidus in experimental animals depend upon the presence of the adeno-hypophysis : removal of this will reduce or abolish the polyuria caused by hypothalamic damage or neurohypophysial destruction. This effect of the adenohypophysis is mediated partly by the thyroid and partly by the adrenal cortex, for removal of either of these glands has much the same effect on experimental diabetes insipidus as removal of the adenohypophysis itself.

Tumours, infections or traumatic lesions in man involving or interfering with any part of the hypothalamic-neurohypophysial complex give rise to the clinical condition of diabetes insipidus in which fluid balance is usually only attained at levels of more than 6 litres per day and the ability to concentrate urine is so poor that a specific gravity of 1·008 is not exceeded. The condition usually resembles the experimental one and responds to treatment with vasopressin given either nasally as powder or by injection in the form of the slowly absorbed tannate. There are, however, reports of unusual cases in which the polydipsia appears to be primary, or in which there is little or no response to vasopressin.

Oxytocin. The evidence for the hormonal *rôle* of oxytocin is not so great as that for the antidiuretic hormone. The separated

oxytocic factor from neurohypophysial extracts is tested by its capacity to cause contraction of the isolated guinea-pig uterus, a capacity which the pressor factor does not possess. The conditions *in vivo* are more complex in that both fractions may have some activity on the uterus and the activity is largely dependent on the hormonal conditions of the animal at the time of the injection. In experimental animals oxytocin tends to promote uterine contraction during periods when œstrogenic activity is predominant and to be relatively inactive in luteal phases. Thus it will not promote contractions during pregnancy but does so at parturition (Stehle, 1950). This is so in pregnant women when the strong contractions of the uterus produced by oxytocin are useful in controlling post-partum hæmorrhage. During early pregnancy the human uterus is insensitive to oxytocin but contracts under the influence of the pressor fraction. Qualitative differences between the effects of the two fractions are possibly due to the predominantly vascular effects of the pressor fraction and the effect on smooth muscle of the oxytocin. One special aspect of oxytocic action on smooth muscle is the stimulus to milk release (" let-down ") in cattle. The bulk of the experimental evidence favours the view that an intact neurohypophysis is essential for normal labour. In its absence dystocia, prolonged labour and even failure of parturition with maceration of the fœtuses have been observed. Uterine contractions have been induced by electrical stimulation of the hypothalamic-neurohypophysial system in rabbits (Harris, 1948). Despite this experimental evidence and the suggestive facts about uterine sensitivity to oxytocin at parturition, there is no clear evidence that the neurohypophysis has a major *rôle* in normal delivery in women.

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THE ADRENAL CORTEX

Introduction. Complete removal of the adrenal cortex is rapidly followed by death. Life can be maintained by injection of extracts of the gland or, more simply, by ensuring a high intake of sodium. The maintenance of electrolyte and water balance in the body is the main

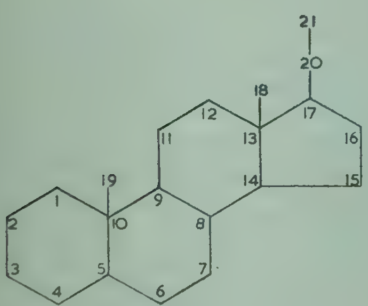
function of the gland but by no means the only one. The other functions have been discovered through clinical studies in cases of adrenal-cortical insufficiency or over-activity and by experimental studies with hormones isolated from the gland.

Adrenal-cortical Hormones. By 1943 twenty-eight steroids had been isolated from the adrenal cortex and crystallized (Reichstein and Shoppee, 1943), and the process continues. Their physiological activity is tested in various ways. The most important, but crudest, method is to determine the amount of steroid required to keep an adrenalectomized rat or dog alive. Other tests have been devised to measure more specific abilities : to prevent muscular fatigue ; to prevent renal loss of sodium and chloride ions ; to prevent potassium retention ; to antagonize insulin ; to foster liver glycogen deposition ; to promote resistance to shock, and so on. It seems unlikely that a single endocrine organ is capable of secreting upwards of thirty hormones, and for this reason we may suppose that many of the steroids isolated are intermediate metabolites formed in the process of elaboration of a much smaller number of actual hormones. When one considers the fact that amorphous material from which the most active of the steroids have been removed may still be more potent than any of the isolated crystalline products, it seems likely that the true hormone is still undiscovered, and this is further reason for considering the crystalline steroids which have been isolated to be nothing more than intermediary metabolites. The rate of hormone secretion into the adrenal vein is very high but the amount of steroid in the gland is quite minute : several kilograms of tissue are required for the isolation of as many milligrams of pure steroid. The simplest classification of the steroids is based on their physiological actions, of which the most important are those influencing water and electrolyte economy, carbohydrate metabolism, and sexual characters. These activities do not exhaust the functions of the adrenal cortex, nor are they mutually exclusive among the steroids so far isolated, but they form a convenient descriptive grouping.

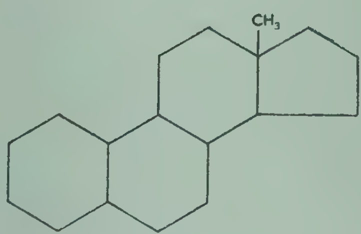
The formulæ of the commonest adrenal and gonadal steroids and of their urinary metabolites most used in diagnosis are pictured on pp. 322, 323. The basic structure is the cyclopentenophenanthrene nucleus—a five-ringed skeleton to which are attached one methyl group, two methyl groups, or two methyl and one ethyl groups to constitute œstrane, androstane, and *allo*-pregnane respectively ; these are the key structures of the

œstrogens, androgens, and corticosteroids. The hydrogen attached at the C_5 position is indicated for *allo*-pregnane and androstane, the dotted attachment indicating that it lies below the plane of the rings —the corresponding isomers in which it lies above the plane are pregnane and ætiocholane ; derivatives of the latter occur in the urine. Since all the œstrogens are unsaturated about this C_5 position the configuration in œstrane is immaterial. The same convention about dotted and full-line attachments applies to univalent attachments to the rings which are called α or β according to whether they lie below the plane or above it.

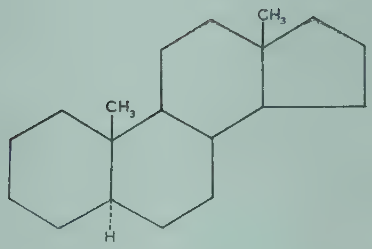
The name corticosterone was given to one of the first physiologically active steroids to be isolated and the others are commonly named as derivatives of this, either by addition of hydroxyl groups or removal of oxygen or hydrogen (hydroxy-, deoxy-, and dehydro-derivatives respectively). The representative steroid affecting the water and electrolyte economy is 11-deoxycorticosterone, chiefly used clinically in the form of its acetate. The presence of an hydroxyl or ketone group at the 11 carbon position is associated with steroids having much less activity in this respect but with greater influence on carhohydrate metabolism. Such steroids are crudely termed 11-oxycorticosteroids and the main representatives, apart from corticosterone itself, are its more active derivatives 17-hydroxycorticosterone (compound F) and 11-dehydro-17-hydroxycorticosterone (compound E or cortisone). Œstrone and progesterone and various androgenic steroids have also been isolated from the gland.



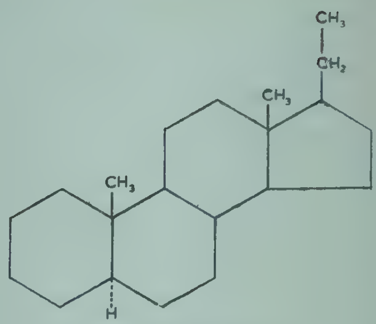
Numbering of the carbon atoms.



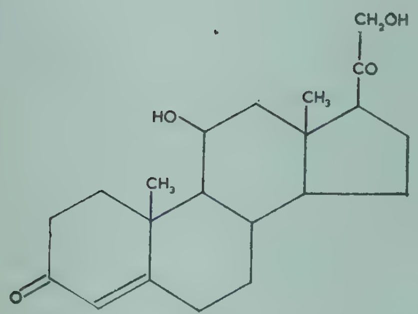
Œstrane.



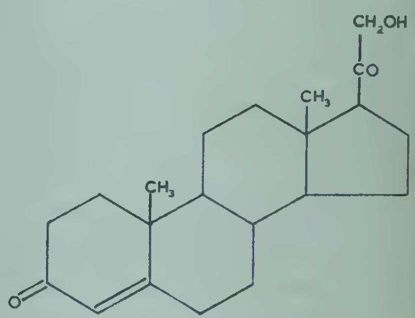
Androstane.



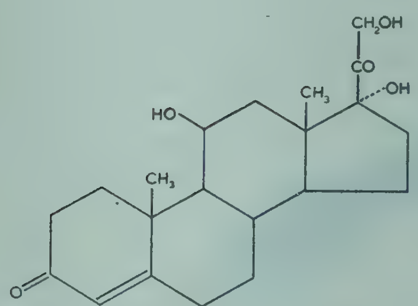
allo-Pregnane.



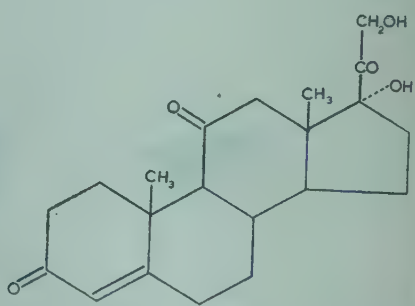
Corticosterone.
Δ_4-Pregnene-11 : 21-diol-3 : 20-dione.



11-Deoxycorticosterone.
Δ_4-Pregnene-21-ol-3 : 20-dione.

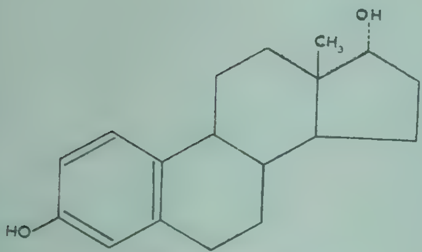


17-Hydroxycorticosterone (Compound F).
Δ_4-Pregnene-11(β) : 17 (α) : 21-triol-3: 20-dione.

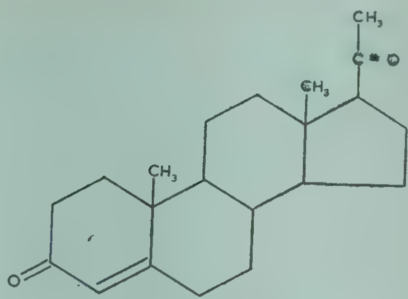


17-Hydroxy-11-dehydrocorticosterone (Compound E or cortisone).
Δ_4-Pregnene-17(α) : 21-diol-3 : 11 : 20-trione.

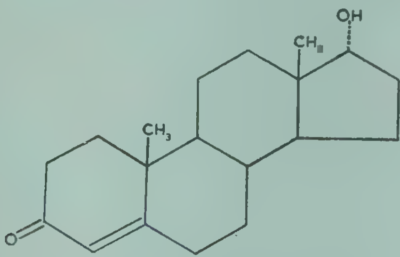
Basic structures and cortical steroids.



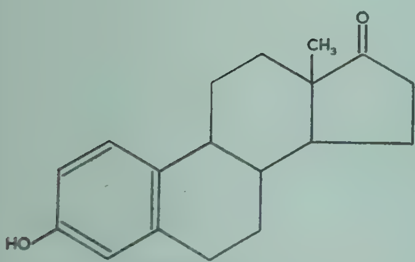
α-Œstradiol.
$\Delta_{1 \cdot 3 \cdot 5}$-Œstratriene-3 : 17(α)-diol.



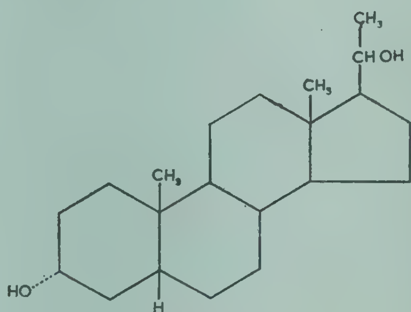
Progesterone.
Δ_4-Pregnene-3 : 20-dione.



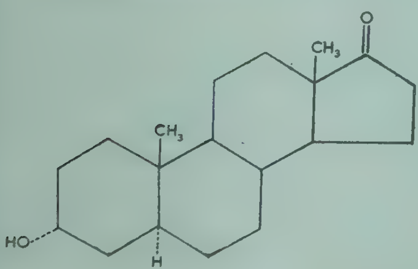
Testosterone.
Δ_4-Androstene-17(α)-ol-3-one.



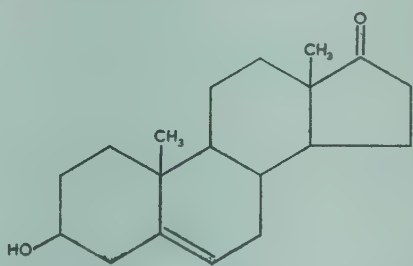
Œstrone.
$\Delta_{1 \cdot 3 \cdot 5}$-Œstratriene-3-ol-17-one.



Pregnanediol.
Pregnane-3(α) : 20(α)-diol.



Androsterone.
Androstane-3(α)-ol-17-one.



Dehydro-*iso*-androsterone.
Δ_5-Androstene-3(β)-ol-17-one.

Gonadal and urinary steroids.

Adrenal-cortical Functions. The outward symptoms preceding death in adrenalectomized animals are loss of appetite, gastrointestinal disturbances, muscular weakness, and prostration sometimes interrupted by terminal convulsions. Growth is stopped in young animals. In the blood there is a rise in the concentration of potassium and non-protein nitrogen and a fall in the concentrations of sodium, chloride and sugar. Capillary permeability is greatly increased. This leads to hæmoconcentration and a reduction in blood volume and is in turn partly responsible for a fall in blood pressure and oliguria. This secondary renal insufficiency may produce the rise in blood urea but there is a primary failure in tubular reabsorption of sodium whilst the reabsorption of potassium is increased. The adrenalectomized animal cannot deal adequately with excess of water ; there is a failure of diuresis and the animal is very prone to water intoxication. Water apparently accumulates within the cells of the body. Most of these changes can be summarized by saying that the distribution of water and electrolytes within the body is deranged, but this should not be allowed to hide our ignorance of the detailed mechanisms involved.

Most adrenalectomized animals can be kept alive almost indefinitely by feeding them on diet containing an excess of sodium chloride and little potassium. By this means the blood electrolyte pattern is restored to normal, a diuresis is induced, which prevents the development of renal insufficiency, and young animals resume growth. Of all changes produced by adrenalectomy, those affecting blood-electrolyte balance are the most formidable and are responsible for most, though by no means all, of its ill-effects. The adrenalectomized animal protected by a high salt intake still has defective kidney function, impaired carbohydrate regulation, muscular incapacity, and extreme sensitivity to stress, and these defects can only be overcome by the injection of cortical extracts or steroids.

In tests based on the ability to maintain life and growth in young adrenalectomized animals the "amorphous fraction" remaining after extraction of the pure steroids is still the most potent product : 11-deoxycorticosterone is the most potent of the pure steroids. There is, however, some doubt whether this steroid can be regarded as a true product of the cortex. It is prepared in practice by chemical means from a plant steroid and though some workers have extracted minute quantities of it from animal adrenals, others have been entirely unsuccessful. As would be

expected from its capacity to maintain life, deoxycorticosterone produces sodium chloride retention and restores normal blood potassium: it also increases blood volume and restores a normal blood pressure. Some of these effects are produced by giving deoxycorticosterone to intact animals and if very large doses are given, or the administration is prolonged for a long period, hypertension or dangerously low blood potassium levels may be produced Polydipsia and polyuria (perhaps due to increased extracellular sodium concentration) together with muscular weakness and incoordination have also been produced.

Deoxycorticosterone and "amorphous fractions" are, however, relatively ineffective in protecting the adrenalectomized animal against stress, in overcoming its muscular weakness, or in restoring its ability to regulate carbohydrate metabolism. These functions of the cortex apparently depend on the 11-oxycorticosteroids, which are much more effective in these capacities. The blood sugar level of the adrenalectomized animal kept alive with salt or deoxycorticosterone may be normal but rapidly falls in the absence of food. This failure of carbohydrate regulation is accompanied by an inability to form liver glycogen. If an 11-oxycorticosteroid is given while the animal is still fasting the blood-sugar level can be restored and liver glycogen formed. There is at the same time an increased urinary nitrogen excretion. This may be supposed to be entirely derived from protein taking part in gluconeogenesis, and on this supposition would account for most of the extra carbohydrate formed. There is, however, some evidence that decreased utilization of carbohydrate is also concerned. Such steroids are also active in tests in which adrenalectomized animals are protected against cold or the stress of swimming.

There are two kinds of muscle-work tests. One measures the amount of work that a muscle can perform when stimulated to contract thrice per second for periods up to forty-eight hours; in this test 11-oxycorticosteroids are much more effective than deoxycorticosterone. In the other form, muscle power is measured for brief periods with plenty of time allowed for recovery between tests, and deoxycorticosterone is found to be the more effective. Evidently different effects are being measured. The 11-oxycorticosteroids are also more effective than deoxycorticosterone in restoring the ability of the adrenalectomized animal to cope with excess water.

The influence of the 11-oxycorticosteroids on carbohydrate

metabolism caused them to be referred to as "gluco-corticoids" by some investigators, but since cortisone has become more freely available for experimental and clinical use the actions of these compounds appear to be so widespread that Ingle (1950) regards the term as akin to "describing an elephant in terms of the shape of its tail."

Regulation of Function. The removal of one adrenal results in hypertrophy of the other only if the adenohypophysis remains structurally and functionally intact. This was the chief evidence for the existence of the hormone adrenocorticotrophin (ACTH) which has now been isolated from the adenohypophysis as a homogenous protein. Removal of the adenohypophysis causes atrophy of the adrenal cortex though, in the rat at least, this only affects the inner parts, the fasciculate and reticular zones; the glomerular zone is relatively little affected. Since hypophysectomy does not cause death, and since injections of deoxycorticosterone are followed by degeneration of the glomerular zone (presumably by disuse atrophy), it is probable that the glomerular zone is responsible for producing and secreting the hormone(s) influencing salt and water distribution, while the fasciculate and reticular zones produce the 11-oxycorticosteroid(s) (Deane and Greep, 1946; Greep and Deane, 1947). The blood in the adrenal vein of dogs will prolong life in adrenalectomized rats but the blood in the adrenal artery or in the general circulation has little or no power to do so. Calculations of the amount of hormone in the vein indicate a relatively enormous difference between the rate of secretion and the amount of hormone normally present in the gland. A 10-kilogram dog will secrete in one day as much life-maintaining activity as can be extracted from about 17 kilograms of adrenal tissue. The output of the cortex assessed in this way is increased by adrenaline (Vogt, 1943–44). Other evidence shows that adrenaline will stimulate adrenal-cortical function *via* the adenohypophysis; the cholesterol and ascorbic acid content of the gland is reduced by a single dose of adrenaline and its lipoid content increased by daily injections, but both these effects are abolished by hypophysectomy (Long and Fry, 1945).

Response to Stress. Selye (1946) has reported investigations in this field. When an animal is harmed by over-exertion, by exposure to heat or cold, by trauma, or by the injection of almost any poisonous substance, the resulting biochemical and pathological changes follow a very similar course, which has been divided into three phases. The first constitutes the shock phase

or *alarm reaction*, followed by a phase of adaptation or resistance to the harmful effects. This eventually merges into an exhaustion phase and death if the alarming stimuli are continued. During the shock phase an immediate outpouring of adrenaline causes an initial rise in blood-sugar level and blood pressure. This reaction is temporary and short-lived and the characteristic hypotension and hypoglycæmia with hæmoconcentration soon develop. Other metabolic changes of varying severity usually produced during the shock phase are a reduction of basal metabolism, ketosis, rises in blood non-protein nitrogen and potassium and falls in blood volume and blood chloride level. Pathological changes include hypertrophy of the adrenal cortex and depletion of its cholesterol, involution of the thymus and lymph nodes, degenerative changes in the pancreatic acini, hæmorrhage and ulceration of the stomach and gut ; fatty infiltration or cloudy swelling of the liver, and œdema of the lung are occasional findings. During the adaptation and resistance phases normal conditions are restored and some of the conditions are even reversed (for instance, a raised blood chloride level, slight hyperglycæmia and hypertension are commonly produced). If the stress is continued then the exhaustion phase reproduces most of the features of the shock phase before death occurs.

The hypertrophy of the adrenal cortex, its loss of cholesterol, and the character of many of the changes that are reversed during the resistance phase suggests that adrenal secretions are implicated. The suggestion is that the harmful stimulus or the changes it produces call for an increased secretion and metabolism of corticosteroids and that this call is mediated by the adenohypophysis. In the absence of the hypophysis or of the adrenals the resistance phase is greatly curtailed. It must be remembered, however, that some degree of resistance can be produced in adrenalectomized animals, showing that the adrenal hormones do not constitute the only bodily defence mechanisms in these circumstances. That increased secretion of corticosteroids is produced in similar conditions in humans is proved by the increased urinary excretion of 17-ketosteroids and corticosteroids during stress or after injury or operation. The relative failure of adrenal extracts or corticosteroids to minimize the effects of shock in intact animals or in humans may be due to inadequate dosage or it may be that the initiation of the shock state is a different process from its continuance.

The whole complex of changes produced by harmful stimuli

has been termed the *general adaptation syndrome* and it has been suggested that analogous changes are responsible for a variety of clinical disorders. The evidence for considering these disorders as diseases of adaptation is based on the production of many pathological changes in animals overdosed with deoxycorticosterone or adenohypophysial extracts. Lesions so produced are vascular changes resembling those of periarteritis nodosa, myocardial and arthritic lesions like those of acute rheumatic fever, nephrosclerosis and nephritis, but these have only regularly been so produced in one laboratory and often there only under peculiar conditions of experiment (with high salt intake or after unilateral nephrectomy). Until the findings have been put on a more quantitative basis and more amply confirmed, their clinical significance must remain in doubt. The changes produced in the general adaptation syndrome constitute only one piece of evidence regarding the relation between lymphoid tissue and the adrenal cortex. After adrenalectomy in animals there is hyperplasia of the thymus and lymph nodes while administration of cortical extracts, and particularly of the 11-oxycorticosteroids, will cause their involution. These changes are accompanied by changes in the blood lymphocytes ; lymphocytosis after adrenalectomy, lymphopenia after administration of adrenocorticotrophin or of 11-oxycorticosteroids in intact animals or of the latter in adrenalectomized animals. A polymorphonuclear leucocytosis may occur despite the lymphopenia. Earlier work suggested that there may be rapid dissolution of lymphocytes, with release of γ-globulins and antibodies into the blood, thus constituting part of a general defence mechanism. Though the release of antibodies in pre-immunized animals has been confirmed, the release of γ-globulins and non-specific antibodies has been denied and cannot be regarded as established.

Clinical Deficiency. Acute adrenal-cortical deficiency may be produced by massive hæmorrhage in the glands—this may occur owing to trauma in new-born infants or as a result of fulminating infections in older children and adults. Chronic insufficiency occurs in Addison's disease. Most commonly there is bilateral fibrocaseous destruction of the glands. Other cases are due to unexplained bilateral cortical atrophy. If only one gland is affected the secretion of the other is sufficient to prevent deficiency symptoms. The symptoms of deficiency are very similar to those produced by adrenalectomy in animals, although, as there are varying degrees of deficiency, many of the changes

may not be apparent until they are revealed by the increased demand for cortical steroids occurring in conditions of stress, infections, etc. Patients usually complain of lassitude and fatigue, gastro-intestinal disturbances (anorexia, vomiting, diarrhœa), loss of weight and pigmentation of the skin. Laboratory investigations reveal the usual symptoms of lowered concentration of blood chloride and sodium, raised blood potassium, hypotension, increased hæmatocrit values, and a reduced urinary output of 11-oxycorticosteroids and neutral 17-ketosteroids. The blood-sugar level may be subnormal but the upset in carbohydrate metabolism is more clearly shown by increasing hypoglycæmia during fasting ; the disease is usually progressive and fatal within five years if untreated.

Life can be prolonged by feeding a diet with a low potassium and high sodium chloride content, but this is not very effective. Better results are obtained by controlling the electrolyte and water disturbances with deoxycorticosterone which is usually effective at a dosage of about 1 mg. per day, most economically achieved by sublingual administration or by pellet implantation, which only need be renewed every four months. In either case no dietary electrolyte control is needed. Such treatment, however, produces little or no improvement in the muscular symptoms and does not enable the patient to withstand any abnormal stress, whilst carbohydrate regulation remains faulty. These symptoms can be controlled by adrenal cortical extracts in very large doses or by cortisone or compound F (Thorn and Forsham, 1949). Such materials are, however, at present scarce or expensive and their use has to be confined to periods of excessive need—as in acute deficiency of pathological or surgical origin, or in Addisonian crises. No treatment appears to affect the pigmentation. As this symptom is not produced by adrenalectomy in animals, investigation of it is difficult and the evidence that the medulla may be concerned is not convincing. Subclinical degrees of cortical insufficiency (as for example in tuberculosis affecting only one adrenal) which can only be demonstrated by tests described later, are perhaps responsible for otherwise unexplained instances of post-operative shock and collapse. It is well established that operations are followed by an increased urinary excretion of 11-oxycorticosteroids and in minor deficiency states an increased production of such steroids might well be impossible. Pre-operative treatment with adrenocorticotropin and administration of cortical extracts during and after the operation

are indicated where such low-grade deficiency has been established.

Clinical Over-activity. Since three types of hormones have been recognized in the adrenal cortex, clinical over-activity might be expected to have very different effects according to which, or how many, of the hormonal functions were affected. This expectation is borne out to some extent in that two chief forms of over-activity are differentiated, one mainly affecting metabolism and the other sexual development. Such a differentiation, however, cannot be pushed to extremes and only indicates which form of over-secretion is predominant. Cushing's disease is generally regarded as involving over-activity of the adrenal cortex though its cause is obscure. The disease usually appears in children, youths or young adults who at autopsy or operation are generally found to have basophil-cell adenoma of the adenohypophysis and/or hyperplasia or tumour of the adrenal cortex ; there are also characteristic hyaline changes with degranulation and vacuolation of the cytoplasm of the basophil cells of the non-adenomatous parts of the pituitary. The preponderance of cases with such pathological changes seems clear enough evidence that some disorder of the adenohypophysial : adreno-cortical system is the basic cause. Cases in which basophil-cell changes are unaccompanied by any histological signs of adrenal-cortical abnormality suggest to some that the disorder originates in the adenohypophysis though others regard the pituitary changes as secondary to a primary over-activity of the adrenal cortex, with suppression of adrenocorticotropin secretion by excess of circulating corticosteroid and eventual disuse atrophy of the basophil cells that produce it. This question can only be settled satisfactorily by assay of the amounts of circulating adrenocorticotropin and cortical hormones. Meanwhile, whatever the ultimate cause of the disease may be, there is no doubt that its symptoms are caused by excessive secretion of adrenal-cortical hormones and that when a tumour of the cortex is present its removal will cure all the symptoms of the disease. The main clinical features of Cushing's disease point to excessive secretion of all types of adrenocortical steroid. The hypertension might be attributed to excessive production of deoxycorticosterone and to support this there is a tendency for the blood potassium level to be low and the blood sodium level to be normal or high. The interference with carbohydrate regulation is shown by hyperglycæmia and reduced glucose tolerance with insulin-resistance. This points to over-production of 11-oxycorticosteroids and

these by increasing gluconeogenesis from protein could account for the wastage of muscle and, by the failure to lay down the protein matrix of new bone, for the osteoporosis. Owing to the osteoporosis there is an increased amount of calcium in the urine which often causes renal calculi. The obesity with its characteristic distribution has been attributed to excess of 11-dehydro-corticosterone (compound A) which appears to increase bodily fat in experimental animals. In spite of these suggestions regarding over-production of multiple hormones it has been found that the very large doses of compound E given to patients with rheumatoid arthritis have sometimes given rise to most of the symptoms of Cushing's disease and certainly to the changes in blood electrolytes. This would suggest that deoxycorticosterone need not be secreted in excess in Cushing's disease and lends slight support to the theory that the disease is adenohypophysial in origin, as the hormone regulating water and electrolyte economy is relatively independent of pituitary control.

Hirsutism in women with Cushing's disease points to over-production of adrenal androgens and this by inhibiting adenohypophysial gonadotropin secretion presumably accounts for the amenorrhœa and impotence which are quite common symptoms ; it is also suggested that excess androgen accounts for the acne and facial flush. The urinary excretion of neutral 17-ketosteroids may be normal or high.

Tumours of the pancreas and thymus have been reported in some cases of Cushing's disease, though their significance is uncertain.

Excessive production of adrenal androgens without the symptoms of Cushing's disease may occur at all stages of life but with very different results. If it occurs before birth in females the whole accessory sex apparatus, which normally in embryo has almost equal male and female potentialities, is directed along the male pathway and a female pseudohermaphrodite is produced, distinguished from a true hermaphrodite by having relatively normal ovaries together with male external genitalia, a masculine voice, muscular development and hair distribution. In males the condition accentuates and accelerates normal sexual and osseous development and pubertas præcox is produced. The equivalent condition occurring in adult life constitutes the adrenogenital syndrome which seems only to occur in women. Possibly the normal range of masculine changes in men embraces the physical possibilities so that excess androgen from the adrenal cortex does

not show itself. In women the masculinization includes hirsutism, amenorrhœa, and degeneration or atrophy of the ovaries and uterus, hypertrophy of the clitoris, deepening of the voice, and development of male musculature and bodily contours. Cushing's disease, and feminine pseudohermaphroditism, and the adreno-genital syndrome may be caused by adrenal-cortical tumours or hyperplasia. If a tumour is present, its removal will dramatically cure the adult conditions and will arrest or cure pseudohermaphroditism, depending on how far this has progressed before the operation is performed. Adrenocorticotropin must be given before operation to stimulate the unaffected adrenal which has usually become atrophic owing to the hormonal activity of the tumour. Cortical extract is given post-operatively. If there is no tumour, only hyperplasia of the glands, treatment is much more difficult and operative interference dangerous. For this reason it is usually reserved for progressive cases of Cushing's disease. Removal of one hyperplastic gland is of little avail, but fairly good results have been obtained by removing all of one gland and almost all the other, although the post-operative course is hazardous. It is usually quiet for a week or two, when there may be a gradual relapse unrelieved by salt or cortical extract. The reason for this is quite unknown, but is possibly connected with some undetected associated abnormality. Some cases of Cushing's disease have responded well, though slowly and often temporarily, to X-irradiation of the pituitary (Thorn and Forsham, 1949). The two chief obstacles in the way of devising a satisfactory treatment of Cushing's disease are the doubts about its cause and its comparative rarity, making it difficult to collect data for the proper assessment of any one treatment. The tumours so far discussed have usually affected more than one cortical function. Other, uncommon, types seem to be more selective. The only symptom of one such tumour was diabetes which disappeared when the tumour was removed. There have also been cases of feminization in men associated with adrenal-cortical tumours.

Tests of Function. No satisfactory test has yet been devised for the routine measurement of the adrenal-cortical hormone(s) in the blood of patients so that the function of the gland has to be assessed clinically by examination of those bodily functions and blood constituents that its hormones are known to influence, or, more indirectly, by measuring those of its products that are excreted in the urine.

There is no doubt that the greater part of the neutral 17-keto-

steroid fraction extractable from urine is ultimately derived from the adrenal cortex, since its rate of excretion is little if at all reduced after ovariectomy in women and only reduced by 20 to 30 per cent. after castration in men. Its excretion is increased by injection of adrenocorticotropin, reduced in Addison's disease but usually increased in cases of Cushing's syndrome, and always in cases of adrenogenital syndrome. The differential diagnosis of tumour and hyperplasia of the adrenal cortex is assisted by study of the nature and amount of the neutral 17-ketosteroids. In cases of tumour their excretion is greatly increased and mainly due to a preponderant excretion of dehydro-iso-androsterone. Since this latter compound is a $3(\beta)$-hydroxyketosteroid, whereas the chief components of the normal 17-ketosteroid fraction (androsterone and ætiocholanolone) are $3(\alpha)$-hydroxy compounds with different chemical behaviour, various separations and differential colour reactions are used to determine the amount or proportion of $3(\beta)$-hydroxy ketosteroids in the total fraction ; if this is large it is strong presumptive evidence of a tumour. Unfortunately, however, as with most diagnosis based on urinary elimination, this test is not universally valid, some cases of tumour excreting no excess of dehydro-iso-androsterone (Allen *et al*, 1950). Owing to the earlier progress in androgen chemistry, methods for the extraction of neutral 17-ketosteroids from the urine are fairly well standardized. The methods for isolation and chemical determination of the 11-oxycorticosteroids are still uncertain though both chemical and biological methods are available. The clinical tests depend on extracting the corticosteroids and estimating the reducing power of the extract (possessed by the short side chain at C17) usually by oxidation with periodic acid leading to the production of formaldehyde which can be chemically estimated (" formaldehydogenic steroid "). Alternatively the extract can be tested by its capacity to promote the formation of liver glycogen in adrenalectomized mice (" glycogenic steroids "). The chemical test evidently includes a large proportion of material that is biologically inactive so that the animal test gives lower results, but the relative values obtained by the two methods are fairly consistent. That the factors measured are derived from the adrenal cortex is proved by their increase in the urine under similar conditions to those that increase the 17-ketosteroid excretion (stress, operation, trauma, adrenocorticotropin injection, and in Cushing's disease) and their reduction in Addison's disease (Callow, 1950 ; Dorfman, 1950).

The changes in the blood electrolytes and in glucose tolerance in Cushing's and Addison's diseases have already been discussed but the functional capacity of the adrenal cortices may also be tested by the injection of adrenocorticotropin or the adenohypophysial-cortical system may be tested by injecting adrenaline or by subjecting it artificially to conditions requiring its full activity and observing other effects besides the excretion of corticosteroids and ketosteroids. The simplest index of the secretion of extra 11-oxycorticosteroids is the blood eosinophil-cell count. This is reduced like the lymphocyte count by 11-oxycorticosteroids but is more clear-cut presumably because the bodily store of eosinophil cells is less than of lymphocytes, so that a fall in their numbers in the peripheral blood is less readily rectified. The eosinophil-cell count normally lies between 100 and 300 cells per cubic millimetre and is carried out in a Fuchs-Rosenthal chamber after dilution with one of several suitable fluids which stain the eosinophil cells and disintegrate the erythrocytes. A low count is almost an indication in itself of adrenal-cortical sufficiency. Four hours after the injection of adrenocorticotropin there is normally a 50 per cent. fall in the count but in cases of cortical insufficiency the fall is less. The same results can be obtained by a subcutaneous injection of 0·3 mgm. of adrenaline, though as this effect is mediated via the adenohypophysis the adrenocorticotropin test may be needed for the rigid exclusion of primary pituitary deficiency. If necessary the result of the test may be finally clinched by observing that adrenal-cortical extract produces a fall in the count (Recant, 1950). The eosinophil-cell count in the adrenalectomized mouse has also been used to test corticosteroid excretion in the urine with some success and attempts are being made to adapt it to apply to the blood.

Another test that has been fairly widely used depends upon the inability of the adrenalectomized animal, or the patient with Addison's disease, to show normal water diuresis. Patients with suspected deficiency are given 20 ml. of water per kilogram in the early morning, having had no fluid during the preceding twelve hours; hourly urine specimens are then collected for five hours. If there is deficiency none of the hourly samples will exceed the overnight (nine-hour) volume of urine collected at the start of the test (Robinson *et al.*, 1941). This test evidently depends on the absence of renal disease but false positive results due to such causes can be eliminated by making a combined urine dilution and urea and chloride clearance tests with appropriate analyses and formulæ.

Tests which are more hazardous, in that they may precipitate a crisis in patients suffering from Addison's disease, depend either on withholding sodium chloride from the diet and measuring the chloride concentration in the urine on the third day after giving a diuretic dose of water, or alternatively on giving a dose of some potassium salt by mouth and measuring the blood potassium level half an hour later. There is a clear rise in cases of adrenal-cortical deficiency (which can be quickly abolished with cortical extract) but no alteration in normal individuals. If the test depending on the eosinophil-cell count proves reliable when it becomes more widely used, the need for these more drastic tests will disappear.

Cortisone and Rheumatism. The first description of the benefits produced by cortisone in cases of rheumatoid arthritis has been followed by a spate of reports only kept in check by the scarcity and expense of the steroid. The treatment was suggested to its originator by the amelioration of arthritic symptoms in situations where adrenal-cortical over-activity might be expected —pregnancy, hepatitis with jaundice, operations, and starvation. By now well over 100 cases of chronic rheumatic disease have been treated with daily injections of cortisone acetate, the average effective daily dose being about 100 mgm. The treatment reduces pain and stiffness and swelling. Some patients become completely symptom-free, but, except in very rare cases, full symptoms recur almost immediately on stopping the treatment. Similar benefits have been reported, though in smaller series, in such allied conditions as psoriatic arthritis, acute rheumatic fever, and gout. The ill-effects that might be expected from over-dosage with cortisone are those of Cushing's syndrome and some of the minor symptoms have already been reported—acne, œdema, hirsutism, striæ formation, muscular weakness and reduced gonadal function. It remains to be seen whether prolonged treatment is capable of totally suppressing the patient's adrenal cortex. The reason for the good results is as obscure as the cause of the arthritis. Cortisone appears to inhibit the proliferation of the cells of fixed mesenchyme. It may be that the osteoporosis of Cushing's syndrome is due, in part at least, to the failure of osteoblastic proliferation to maintain normal bone structure. The local cellular response of acute and chronic inflammations in cortisone-treated animals is suppressed and this seems to be accentuated when the inflammation is associated with bacterial hypersensitivity. Healing by granulation is suppressed in the same way. It may be that this mysterious inhibition of cell growth in reacting

mesenchyme may prove to afford some explanation of the temporary amelioration of the symptoms of rheumatoid arthritis.

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THE OVARIES

Function. The endocrine function of the ovary is to secrete œstrogen and progesterone which are necessary adjuncts to its function of producing ova. Both the endocrine function and the maturation and shedding of ova are under the control of the adenohypophysis ; after hypophysectomy there is atrophy of the ovary and sex organs and failure of sex function. This function is cyclic in all female mammals though the extent and nature of the cycle varies enormously in different species.

Œstrous Cycle. In most female animals sexual life can be divided into a phase of heat (œstrus) when they are willing to mate, and phases of quiescence (anœstrus), pregnancy, or pseudopregnancy during which they show no sexual interest and resist mating. Some animals such as bitches and most farm animals only show œstrous phases seasonally and not very often during the year. The commonest laboratory animals show little seasonal variation in breeding capacity and either have regular œstrous cycles like the rat or an almost constant state of œstrus as in the rabbit. In the virgin rat œstrus occurs regularly every four to five days and is accompanied by spontaneous ovulation. A fresh batch of ten or twelve follicles have become swollen with fluid during the four to five days since the previous ovulation and this follicular swelling coincides with an accumulation of fluid in the uterus and proliferation of the vaginal mucosa. After ovulation the fluid soon disappears from the uterus and cornified cells are sloughed off the

vaginal walls before the development process is renewed. None of these changes occurs in the absence of the ovaries but the uterine and vaginal changes can be reproduced in spayed rats by injecting œstrogen extracted from the ovaries or synthesized in the laboratory. The coincidence of the vaginal and uterine changes with the swelling of the follicles, and the presence of high concentrations of œstrogen in the liquor folliculi, suggested the follicles as the source of œstrogens. Cytochemical evidence points to the theca interna as the main site of the formation of ovarian œstrogen, though probably not the only one for cyclic activity may persist after destruction of all follicles and oocytes by X-irradiation of the ovaries. The corpora lutea that form after each set of ovulations in the virgin rat are short-lived and soon regress, making way for the swelling follicles and new corpora lutea of the next œstrous phase. If the rat is allowed to mate the corpora lutea persist and secrete progesterone which prepares the uterus for the implantation of the fertilized egg and the formation of the placenta. In the rat the histological changes in the endometrium are not very striking but are much more so in the rabbit. If either animal is spayed or has its ovarian corpora lutea removed after mating the progestational changes in the uterus are not produced nor is implantation possible ; these occur quite normally if luteal extract or progesterone is injected.

The longer survival of functional corpora lutea in the rat and the initial progestational changes that occur in the uterus of the rat and rabbit after mating do not depend on the success of the mating and can be induced by mechanical or electrical stimulation of the cervix during œstrus or by mating the females with vasosectomized bucks. This condition of pseudopregnancy lasts for ten to twelve days in the rat and for fifteen days in the rabbit (the normal gestation periods are twenty-one to twenty-two and twenty-eight days respectively) before it regresses and œstrus recurs. The secretion of progesterone by the corpora lutea during pseudopregnancy is suggested by their histological appearance and proved by the progestational proliferation of the uterus in the rabbit and by the formation of deciduomata if the uterus is traumatized in the rat. (Deciduomata are endometrial overgrowths simulating placenta formation.) In the absence of hypophysis, œstrous cycles, ovulation and pseudopregnancy can all be produced by injection of pituitary extracts which have been separated into three pure proteins : follicle-stimulating gonadotropin, luteinizing gonadotropin and luteotrophin (prolactin). The first two produce growth of the follicles, œstrogen secretion, ovulation and luteinization in mature follicles, while the third is necessary to sustain progesterone secretion by the formed corpora lutea.

Menstrual Cycle. The sex cycle in man and primates has analogies with that in the lower animals and may be considered as a cycle in which ovulation and pseudo-pregnancy are both spontaneous while sexual receptivity is almost entirely removed from endocrine control. The increasing œstrogen secretion during follicular swelling is reflected in proliferation of the endometrium during the first half of the cycle after menstruation has stopped. The bursting of one follicle and the formation of a corpus luteum starts the secretion of progesterone and secretory changes in the endometrium which are suddenly terminated after about

fourteen days by a sudden regression and sloughing of the developed endometrium during menstruation. These endometrial changes can be duplicated in ovariectomized women by a course of œstrogen injections followed by injections of œstrogen plus progesterone ; on stopping the injections menstrual bleeding is produced from a secretory endometrium just as in the normal cycle.

Diagnostic Methods. Œstrogen and progesterone have been detected in blood but the methods are too elaborate and specialized to be available for other than experimental use, so that study of ovarian function has to depend on study of urinary secretion and of the effects of the hormones. The œstrogen that has actually been isolated from the ovary is α-œstradiol but œstrone and œstriol are present in women's urine. The natural œstrogens have the same ring structure as the adrenal steroids but differ in having no side chain at C_{17} nor a methyl group at C_{10} (i.e. they have 18 carbon atoms) and by the fact that the A ring is trebly unsaturated and, having a hydroxy group at C_3, behaves as a phenol. This renders the œstrogens soluble in alkali and so separable from neutral 17-ketosteroids. In the urine most of the œstrogen is in combined form either as glucuronidate or sulphate, so that the urine has first to be hydrolysed to free the œstrogen before extraction ; when it has been taken up into alkali (sodium hydroxide or bicarbonate solution) it can then be estimated colorimetrically or biologically. The colorimetric methods are much simpler than the biological ones but are only accurate for larger quantities of œstrogen than are excreted during the menstrual cycle. Separation of the urinary œstrogens into œstradiol, œstrone, and œstriol fractions is possible, but investigation of variations in the proportions excreted is still experimental and the significance debatable. The matter is obviously important since a small increase in secretion of œstradiol will affect the biological assay much more than similar increases in the less active œstrone or œstriol. Progesterone is metabolized into pregnanediol before it is combined with glucuronic acid to be excreted as pregnanediol glycuronide or sodium pregnanediol glucuronidate (this somewhat confusing distinction in nomenclature between the free ester and the sodium salt is becoming usual in practice). This substance can be extracted from women's urine in solid form and weighed or colorimetrically estimated. Again the amounts excreted during the normal cycle are at the lower limits of accuracy of the chemical methods (Haslewood, 1950). Very little urinary œstrogen and pregnanediol is excreted during the normal menstrual cycle and there is great variation

between individuals and in day-to-day rates of excretion in the same individual. It is also salutary to remember that when œstrogen or progesterone is injected into women only about 10 per cent. can be recovered from the urine. Œstrogen is usually detectable throughout the cycle, but rises to a peak at mid-cycle, coinciding with ovulation, and is higher in the luteal phase of the cycle than in the preceding proliferative phase. There is also a tendency for œstrogen excretion to show a second peak during the luteal phase, but this is less sharp and lower than the ovulation peak. Pregnanediol is not detectable during menstruation or the proliferative phase of the cycle but is found during the mid-parts of the luteal phase, that is when the corpus luteum is functional. The changes in the endometrium during the menstrual cycle can be followed by taking biopsy specimens. During menstruation the endometrium degenerates and is shed so that when bleeding stops it may be only 0·5 mm. thick. The epithelium regenerates rapidly and the endometrium grows thicker with lengthening of straight tubular glands and plentiful mitoses. This proliferative or follicular phase terminates at ovulation, after which the glands continue to grow but also become coiled and distended with secretion and the glandular cells increase in height. During this secretory or luteal phase decidual cells appear in the hyperæmic stroma and the endometrium may attain a thickness of 5 mm. ; this phase terminates in menstruation. A further indication of ovarian function is based on basal temperature changes. The morning rectal temperature in the absence of infection is lower by about 0·8 degrees during the follicular phase of the cycle than it is during the luteal phase and ovulation can often be timed by a drop in morning temperature of 0·2 to 0·4 degrees for one day which indicates the start of the luteal phase (Davis, 1946). Similar temperature patterns can be induced in women after the menopause by giving œstrogen for several days and then œstrogen plus progesterone though in this case the slight drop in temperature preceding the rise due to progesterone is not seen.

Disturbance of Function. Congenital absence or lack of development of the ovaries (ovarian agenesis or Turner's syndrome) is naturally associated with an absence of the bodily and genital changes of puberty, as well as with a variety of skeletal abnormalities. The contrary condition of precocious puberty is sometimes seen as a normal process apart from the precocity but is more commonly pathological and due to granulosa-cell tumour of the ovary unaccompanied by ovulation and with quite irregular

uterine bleeding and high urinary œstrogen excretion. Amenorrhœa is naturally present before puberty and after the menopause when there is a gradual failure and atrophy of the ovaries with consequent atrophy of the breasts and genital organs. When it occurs during the sexually active years its origin may be ovarian or hypophysial, or it may be due to a failure of the complex inter-relations between ovarian and hypophysial hormones that are responsible for maintaining the normal cycle. Treatment of amenorrhœa, where no pathological cause such as polycystic ovaries or ovarian tumour is present, is largely empirical owing to the difficulty of diagnosing the primary cause of the disorder. Normal menstruation can usually be reproduced by properly spaced treatment with œstrogen and œstrogen plus progesterone and sometimes such treatment will successfully start a renewal of normal cycles. Excessive bleeding from the uterus can also have a variety of causes and can to some extent be controlled by empirical treatment with œstrogen or progesterone, or gonadotropins. Both excessive bleeding and amenorrhœa may be caused by one or other of the tumours that occur in the ovary. Granulosa-theca-cell tumours usually secrete œstrogen; luteomas, a rare luteinized form of granulosa-cell tumour, secrete progesterone; arrhenoblastomata secrete androgens; and tumours of adrenal-cortical rests secrete androgens and corticosteroids.

Lack of ovulation can occur in apparently normal cycles, as menstruation occurs in proliferative endometria when œstrogen concentration is reduced, just as in secretory endometria it occurs when progesterone concentration is reduced. Both these forms of menstruation have been hormonally induced in spayed monkeys and post-menopausal women; a proliferative endometrium developed under œstrogen treatment breaks down with uterine bleeding six to ten days after stopping the treatment (œstrogen-withdrawal bleeding); if progesterone is given during œstrogen treatment so that a secretory endometrium is formed, then menstruation occurs two to three days after stopping the progesterone injections whether or not the œstrogen injections are stopped at the same time (progesterone-withdrawal bleeding). Œstrogen injections started in the secretory phase of the cycle in normal women will inhibit ovulation and postpone menstruation for considerable periods while the injections are continued, but if the injections are begun after ovulation has occurred menstruation occurs at the normal time.

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PREGNANCY AND LACTATION

Placental Hormones. The corpus luteum formed at ovulation starts to regress after about ten days, pregnanediol excretion falls and menstruation soon follows. If the ovum is fertilized the corpus luteum does not regress but expands and hypertrophies, pregnanediol excretion continues and gradually increases and menstruation does not occur. These changes coincide with the formation of the trophoblast. This secretes chorionic gonadotropin which soon attains a high concentration in the circulation and appears in the urine, where its detection is the basis of the Aschheim-Zondek, Friedman and other pregnancy tests. The function of this hormone is to act as a luteotrophin to maintain the structure and secretory function of the corpus luteum. In monkeys injections of chorionic gonadotropin started during the luteal phase of the cycle will delay the onset of menstruation for ten to fifteen days but no longer ; menstruation when it does occur is from a progestational endometrium. At this time (about twenty-five days after ovulation) morphological signs of regression occur in the corpus luteum of pregnancy (Hisaw, 1944). Spaying monkeys before this time terminates pregnancy but does not if it is carried out later. Menstruation in women can also be delayed by injections of large amounts of chorionic gonadotropin (10 to 20,000 i.u. daily) started in the luteal phase of the cycle (Bradbury *et al.*, 1950). Evidently the luteotrophic action of chorionic gonadotropin merely serves to tide over the period until the placenta itself can secrete sufficient progesterone to maintain pregnancy without any contribution from the ovary.

The excretion of chorionic gonadotropin continues throughout pregnancy but at a very low rate in comparison with the high rate of excretion during the first two months. Meanwhile the urinary excretion of œstrogen and pregnanediol increases slowly during the first trimester, then more rapidly, and attains daily rates of about 60 mgm. of œstrogen and about half as much pregnanediol in the last trimester, though the values in different individuals vary greatly. The urinary excretion of more than 6 mgm. of pregnanediol per twelve hours has been used as a pregnancy test but the individual variations make it a less accurate one than those based on gonadotropin excretion. The urinary pregnanediol excretion is also useful in the prognosis of threatened abortion, which is usually associated with low or decreasing rates of excretion indicating some failure of placental function. When the excretion is subnormal the prognosis is bad and the embryo is often already dead ; with normal but decreasing values the prognosis is usually better. The benefits to be derived from progesterone treatment are the subject of statistical debate ; it is obviously difficult to evaluate the results of treating a complication which is threatened rather than actual. There is also the consideration that the primary failure in abortion is unknown and that progesterone lack is as likely to be a symptom as a cause. The excretion of both œstrogen and progesterone usually declines during

the few days immediately before parturition and both reductions have been suggested as causing the onset of labour. Large doses of œstrogen in women and monkeys, however, have not prolonged gestation though this can be achieved with progesterone in some species. On this view delivery might be looked upon as a form of super-menstruation initiated by falling progesterone concentration. Even if this were a true analogy the factors controlling the duration of function of the placenta would still be unknown. It has been shown by experiment in laboratory animals that destruction of the fœtuses in early pregnancy need not interfere with the continued normal life of the placentas which persist for the normal duration of pregnancy before being expelled as at normal parturition.

Mammary Development. Species difference in the hormonal control of mammary development make generalization of the matter difficult. That the development depends chiefly on ovarian hormones is obvious from the lack of mammary development before puberty, the rapid development at that time and regression after ovariectomy or the menopause. The developed mammary gland of the non-pregnant woman is essentially a compound tubular gland with little or no alveolar development ; the alveoli develop rapidly only during the latter part of pregnancy. This is in agreement with the findings in most animals that the ducts develop under œstrogenic stimuli but that progesterone is needed for alveolar development (both hormones are found in the adrenal cortex and this may explain the tubular-alveolar development produced by either alone in some experiments). These appear to be direct effects and can be obtained by local application of the steroids to the breast in spayed animals. The necessity for the presence of the hypophysis is of debatable significance. While some authors claim that specific adenohypophysial hormones are necessary for mammary development, others attribute failures to produce this with steroid hormones in hypophysectomized animals to non-specific effects of the operation. The hormones producing mammary development during pregnancy in experimental animals are mainly secreted by the placenta. This is proved by the rapid mammary regression that follows removal of the placentas in comparison with the minor effects of removing the fœtuses, ovaries, or hypophysis.

Lactation. In non-pregnant animals with mammary glands developed by œstrogen and progesterone it has been possible to initiate milk secretion by injecting adenohypophysial extracts, and study of the stimulating effects of such extracts on the crop-gland of the pigeon eventually led to the isolation of a pure lactogenic hormone (prolactin). The secretion of this hormone from the pituitary is apparently necessary for the initiation of milk secretion. There is some suggestion that its secretion is inhibited by œstrogen in excessive amounts such as are present in the body in the weeks before parturition, an interpretation used to explain the inhibition of lactation produced by œstrogen treatment in the puerperium. This, however, is disputed in that it is claimed that though the pain of breast engorgement is relieved, the treatment does not suppress lactation if nursing of the infant is continued, certainly lactation cannot be inhibited once it is established. The fact that full mammary development and lactation has been

induced in virgin goats and heifers treated with œstrogen alone may be relevant despite the species difference. At the same time, this effect is rather uncertain and apparently depends on correct dosage within a rather narrow range. It has been suggested that œstrogen stimulates the pituitary gland in certain doses and inhibits it with higher ones. Local application of prolactin to isolated parts of the mammary duct system in pregnant or pseudo-pregnant rabbits has shown that the hormone stimulates mammary growth as well as producing milk secretion. The disappointing results of prolactin administration in women with poor lactation suggest that deficiency of the hormone is rare. Milk secretion, once established, is largely maintained through suckling stimuli passing *via* the nervous system to the hypothalamus and hypophysis. This nervous stimulation in the cow provokes a secretion of neuro-hypophysial oxytocin which contracts the musculature of the gland so that its emptying is more complete (" let-down "). Suckling stimuli also increase the rate of prolactin formation by the adenohypophysis in experimental animals. Once suckling stimuli cease the mammary gland soon regresses to its non-pregnant condition, but so long as it is continued milk production continues, even, in such animals as the rat, throughout a concurrent pregnancy, thus showing clearly that the inhibitory stimuli that have been postulated to explain the postponement of lactation from the fully developed gland until after parturition have no effect on already established lactation.

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THE TESTES

Androgen. The effects of androgens on fat distribution, muscular development, the vocal cords, the genitalia, on the prostate, seminal vesicles, bodily hair and other secondary sex characters have been thoroughly investigated in man and experimental and farm animals, though the regulation of sex function has not received so much attention in males as it has in females. The only androgen that has been isolated from the testes is testosterone, but other less active androgens (androsterone and dehydroandrosterone) are excreted in the urine by men and women. All these compounds are neutral 17-ketosteroids and can be colorimetrically or biologically assayed in urine extracts. The chemical methods usually give results indicating a greater quantity of neutral 17-ketosteroids than can be detected by biological assay but there is usually a quantitative relation between the two results which allows the easier chemical method to be used for routine purposes (Callow, 1950). The value of the 17-ketosteroid excretion rate in diagnosis of testicular function is lessened by the fact that castration only causes a 20 to 30 per cent. fall in the rate, and injections of testosterone produce a rise that is on the average only equivalent to about 10 per cent. of the injected material. Evidently most of the urinary 17-ketosteroids are

of adrenal origin and only a small fraction of the androgen produced by the testes appears in the urine. The source of the testicular androgen is the intertubular Leydig cells. Normal androgenic function can be maintained in cases where the seminiferous tubules are completely atrophic, while tumours of these cells result in precocious puberty and increased 17-ketosteroid excretion. Androgens not only specifically stimulate the growth of the sexual organs but are also partly responsible for such secondary sexual characters as the differences in bodily habit. Androgens stimulate general anabolism. Following their administration there is retention of nitrogen, potassium, sodium, phosphates and water, together with a gain in weight not only in the pre-puberty period and in castrated or eunuchoid individuals but also to a lesser extent in normal adults (Kochakian, 1947). Androgens have proved to be useful in the clinical treatment of Addison's disease. In experimental animals the nitrogen retention and protein anabolism can be related to the development of certain muscles which are normally more developed in males than in females. The lesser nitrogen retention in adults suggests that the effect is normally a limited one chiefly concerned with development during puberty. The effects of androgen on growth in general are illustrated in the abnormal growth of children with masculinizing tumours and the dwarfing or retarded bone growth seen in pre-pubertal eunuchs.

Control of Function. As with the ovary, the control of the two functions of the testis (spermatogenesis and androgen production) depends upon the adenohypophysis, removal of which in immature animals prevents all pubertal changes or in adults causes atrophy of the sex organs. The separate function of two gonadotropins is also probable though not quite so clear as in the female. The luteinizing hormone stimulates androgen production and consequent growth of the accessory sex organs. Androgens themselves directly stimulate spermatogenesis in hypophysectomized animals : luteinizing hormone can then restore complete normal function after hypophysectomy. Since pure follicle-stimulating hormone will also stimulate spermatogenesis the contribution of the two hormones to normal testicular function is not clear. The urinary gonadotropin excretion in man or woman is measured by extraction and biological assay which usually gives a crude estimate of total gonadotropic potency with no indication of the relative contribution of follicle-stimulating and luteinizing activities. The results of assays are of uncertain significance since varying proportions of the two activities completely alter the dose : response relations on which the tests are based, a very small alteration in relative proportions may falsely suggest a considerable variation in total excretion. This ill-defined total gonadotropin excretion in men increases with age. Large doses of androgen will suppress this secretion, but in animals, and probably in man, too, this suppression is only achieved with unphysiologically high doses. The cytological changes and increased gonadotropin content produced in the adenohypophysis by castration are only remedied by doses of androgen much larger than are required to restore normal development of the prostate gland and seminal vesicles. Eunuchoid symptoms in man can be controlled by testosterone without lowering the high gonadotropin excretion though this can be lowered with higher

doses of androgen (Heller and Nelson, 1948). These facts have led to the suggestion that the mutual regulation of function by the adenohypophysis and the testis is effected by a second testicular hormone produced by the Sertoli cells, and that this is an œstrogen (Howard *et al.*, 1950). Cytochemical tests indicate that these cells contain lipid material like the Leydig cells and at least one case of Sertoli cell tumour with hermaphrodite features due to œstrogen secretion has been reported.

Puberty. The obvious cause of the onset of puberty is that there is then a rapid increase in androgen secretion. Experimental work casts some doubt on this conception. The urinary excretion of 17-ketosteroids shows a fairly steady rise during the first two decades of life with no sudden increase at puberty. This could be due to an increased production at the time but the androgen content of bull's testis shows a similar steady rise without any acceleration at puberty. The explanation may lie in the observation that the prostate gland and seminal vesicles in rats castrated soon after birth show a sudden increase in sensitivity to androgen at the age when puberty would normally occur. This suggests that puberty is due to a sudden increase in responsiveness of the tissues rather than to increased androgen secretion (Hooker, 1948).

Testicular Deficiency. The main difficulty in diagnosis of testicular deficiency is in deciding whether the condition is primarily gonadal or hypophysial. Decisions are helped by studies of gonadotropin excretion, 17-ketosteroid excretion and examination of semen samples and testicular biopsy specimens. Cryptorchidism is a testicular disorder that can be diagnosed in infancy—the testes retained in the abdomen or inguinal canals are kept at a higher temperature than in the scrotum and spermatogenesis is incomplete under these conditions. Androgen production is usually unaffected and the testes descend at puberty in most cases. The high proportion of spontaneous cure at puberty makes evaluation of hormonal treatment difficult ; chorionic gonadotropin has been claimed to produce descent by stimulating the Leydig cells before puberty ; androgen treatment merely produces premature puberty. The age at which puberty occurs normally shows great individual variation and delayed puberty is often an extreme example of the normal rather than a pathological condition. If delayed puberty is associated with dwarfing then the adenohypophysis is probably responsible but where height is about normal, with a tendency for the length of legs to exceed that of the trunk and head, primary gonadal failure with delayed closure of the epiphyses is the more likely cause ; the urinary gonadotropin excretion is usually higher than normal in such cases.

Androgen deficiency in youths or adults can be treated with

testosterone injections or pellet implants, or by methyl testosterone absorbed sublingually all of which will restore to normal the size of the genitalia and develop the secondary sex characters. Many infertile men show defective spermatogenesis with apparently normal Leydig cells and androgen secretion. Efforts to restore or increase spermatogenesis by hormonal means have not been very successful, though some cases have responded to androgen. Treatment with gonadotropins has been chiefly confined to human chorionic gonadotropin or serum gonadotropin from pregnant mares ; both give rise to antihormones and have had only limited clinical success. It may be that satisfactory treatment of male infertility due to failure of spermatogenesis will be possible when purer hypophysial gonadotropin preparations are available.

Some authors have described cases of " male climacteric " in elderly men in which declining 17-ketosteroid excretion has been accompanied by some symptoms like those of women at the menopause, such as hot flushes. Urinary gonadotropin excretion tends to be high and testicular biopsy shows a decrease in spermatogenesis with peritubular fibrosis and reduction in the number of Leydig cells.

Prostate Pathology. The benign hyperplasia of the prostate that is common in elderly men is difficult to reconcile with the decreasing androgen secretion that is common in old age and it has been suggested that it is due to unantagonized œstrogenic action, though without any very convincing direct evidence. Prostate cancer, on the other hand, is definitely fostered by androgen and its course can be delayed, though not prevented, by castration or by suppressing adenohypophysial gonadotropic activity with œstrogen treatment.

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THE THYROID GLAND

Introduction. The original proof that the thyroid was an endocrine gland was based almost entirely on clinical evidence. When iodine was discovered a clinician soon realized that this was the active agent in such remedies as burnt sponge which had been used in the treatment of

goitre since mediæval times. The syndrome of myxœdema was then described and correlated with thyroid atrophy or absence, and finally, a clinician successfully treated the condition with extracts from sheep thyroid. Meanwhile chemical work had demonstrated the inverse relation between the goitre incidence in a locality and the iodine content of its water, and the peculiar connection between iodine and the thyroid was explained when thyroxine was eventually isolated and synthesized and proved to be an iodine-containing compound (Harington, 1947).

The clinical findings are easily confirmed by animal experiment. Removal of the thyroid inhibits all growth and maturation in young animals and causes a lowering of the rate of general body metabolism at all ages, whilst these effects can all be overcome by injecting thyroxine. The function of the gland is to collect and concentrate iodine from the blood and to synthesize thyroxine in the epithelial cells of the follicles. This thyroxine may be secreted directly into the blood or be stored in the follicular colloid to be re-extracted later by the epithelial cells and then secreted. Whether secretion or storage occurs depends on the demands of the body. These are conveyed to the thyroid *via* the adenohypophysis which secretes thyrotropin at a variable rate depending on the amount of circulating thyroxine. The simplest demonstration of this common type of mutual endocrine regulation is by using antithyroid drugs which inhibit the synthesis of thyroxine by the thyroid. When such a drug is given the thyroid gland becomes hyperplastic and loses colloid and exactly resembles the gland of an animal given excessive amounts of thyrotropin. If thyroxine is given at the same time as the antithyroid drug, then the thyroid remains completely normal. None of these effects is produced in the hypophysectomized animal for the thyroid becomes atrophic and remains so despite the administration of antithyroid drugs (Astwood and Bissell, 1944).

Thyroxine deficiency may be produced in a variety of ways. It may be due to lack of the iodine which is an essential part of the thyroxine molecule ; it may be caused by a functional deficiency of the thyroid gland or adenohypophysis ; or it may be produced by over-zealous surgical or medical treatment of hyperthyroidism when too much of the gland is removed or too large amounts of antithyroid drugs are administered. Although the broad effects of thyroxine deficiency are the same, the course of the disorder varies somewhat according to the age at which it occurs and its cause.

Endemic Goitre. In districts where the water contains subnormal amounts of iodine, usually, but not always, in regions far from the sea, the diet may not provide enough iodine to maintain normal thyroxine synthesis. The consequent decrease of thyroxine in the body stimulates an increase in thyrotropin secretion and this induces growth and hyperplasia of the thyroid gland which continues until the enlarged gland, by its more efficient use of the available iodine, is able to restore a normal rate of thyroxine

synthesis and of bodily metabolism, but this may not be achieved until a large goitre has developed. The hyperplasia usually does not persist, and, either because the supply of iodine becomes greater or the needs of the body less, involution leads to the formation of large colloid-filled follicles (colloid goitre). If extra iodine is given early enough the goitre may recede by that means alone but surgery is usually necessary once the goitre has become large. If the hyperplasia is allowed to continue, exhaustion atrophy will take place. The atrophic glandular tissue is replaced by fibrous tissue which often becomes calcified with the passage of time. The surviving glandular tissue subjected to continuous thyrotropic stimulation undergoes progressive hyperplastic enlargement producing the familiar picture of a nodular goitre. However, exhaustion atrophy may proceed so far that hypothyroidism is produced. Transient goitres sometimes appear at times when there is a temporarily increased demand for thyroxine by the body which is not catered for by the ordinary iodine intake. Goitres may occur thus at puberty or during pregnancy but disappear as soon as iodine is given or the period of excessive demand ends.

Hypothyroidism. Myxœdema, the syndrome caused by hypothyroidism in adults, is usually caused by exhaustion atrophy of a simple goitre or by too drastic treatment of hyperthyroidism by surgery or antithyroid drugs. It may occur also as a result of a failure of adenohypophysial thyrotropin secretion but this is seldom found as a single condition, myxœdema of such origin usually being only one aspect of the wider disorder of Simmond's disease. In either case the basic disturbance is a lowering of the rate of general metabolism and almost all the symptoms are secondary to this. If thyroid failure occurs in a fœtus or child the most prominent symptoms are lack of skeletal development and of maturation—the myxœdematous symptoms are not so pronounced as in adult cases. The usual cause of the thyroid failure seems to be an acute infection—in the mother when the condition is congenital. In the past the most usual form was that occurring in endemic goitre areas where the mother's iodine lack was aggravated by the pregnancy; such cretins are particularly difficult to treat, in contrast to the other types in whom thyroid medication, if started early enough, is fully capable of producing almost normal growth and maturation. So long as treatment is continued the patients are freed from hypothyroid symptoms as are adult cases of myxœdema similarly treated.

Hyperthyroidism. Excessive secretion of thyroxine (Graves' disease or thyrotoxicosis) causes an increase in metabolic rate and the opposite secondary symptoms to those of hypothyroidism. Exophthalmos is a common associated symptom. The thyroid gland is enlarged to form a goitre with the histological features of over-activity, the follicles being small, they contain little or no colloid and their epithelium is hyperplastic and thrown into projecting papillary processes. These effects suggest that the disease may not be primarily a thyroid disorder but that it is caused by excessive production of adenohypophysial thyrotropin. Satisfactory treatment or cure of thyrotoxicosis is difficult while its ultimate cause is uncertain. If the disorder originates in the hypothalamico-hypophysial system, then treatment at this site should be preferred and in fact some cases have responded to X-irradiation of the pituitary gland. Such an effect, however, is rare and the treatment is inevitably dangerous to the other pituitary functions. It is doubtful if the proportion of cures by such means is greater than that of spontaneous remissions—the latter figure is, of course, impossible to assess accurately. Treatment, therefore, chiefly aims at preventing the excess thyrotropin being effective by hindering its action on the thyroid gland. This is done by giving iodine or antithyroid drugs or by destruction of the greater part of the goitre by surgical removal or by radiation applied locally by isotope. The relative efficiences of these methods is debated and greatly depends on the characteristics of the individual case.

Exophthalmos. Protrusion of the eyeball has been produced in thyroidectomized guinea pigs by the injection of thyrotropin, the protrusion being due to deposition of fat and œdema of the orbital tissues. Unfortunately thyrotropin is one of the hypophysial hormones that has not yet been purified and there is some evidence that a contaminating factor may really be responsible for this effect in experimental animals. Exophthalmos in Graves' disease varies considerably in onset and occurrence. It may precede or follow the development of the other hyperthyroid symptoms, or these may develop without there being any exophthalmos. It also varies in intensity. The milder form shows lid retraction, protrusion of the eyeball and stare, all of which usually regress when thyrotoxicosis is treated. The more severe form (malignant exophthalmos) is accompanied by swelling of the lids (which are not always retracted), the measured protrusion of the eyeball is greater, and ocular movement may be limited and

eventually arrested by damage to the ocular muscles ; this type of exophthalmos is often exacerbated by treatment of the thyrotoxicosis. It is convenient to consider the mild form as being caused by excess thyroxine and the severe form as being caused by excess thyrotropin (or associated adenohypophysial factor) ; this would explain the exaggeration of the symptoms of the latter by treatment which may be presumed to release the adenohypophysis from the inhibitory effect of circulating thyroxine and certainly the orbital changes in this form of exophthalmos are like those produced by thyrotropin in experimental animals. No experimental exophthalmos has, however, been produced by thyroxine (Dobyns, 1950).

Methods of Diagnosis. As the control of basal metabolism is the chief function of the thyroid hormone, its measurement is a logical and direct measure of thyroid function. The rate of metabolism is determined by measuring the oxygen uptake under controlled basal conditions and expressing the result as a percentage of the normal value for individuals of the same age, sex and body surface. Such a measurement is moderately simple to make and can be performed in the out-patient department if the patient is co-operative. The interpretation of the result, particularly with borderline values, must take the whole range of symptoms into account because of the variation in normal values. For instance, an individual with a naturally low rate of metabolism may have clinical hyperthyroidism though his basal metabolic rate may lie below the upper limit of the normal range. Another direct method of measuring the thyroid function would be to determine the level of circulating thyroxine but such a procedure is not yet possible as a routine. A method that approximates to the estimation of the blood thyroxine is the determination of the protein-bound iodine in the blood, a difficult but feasible procedure. All the thyroxine in the blood is contained in this fraction and under normal circumstances it is the only variable constituent. Normally the blood contains 4 to 8μg. of protein-bound iodine per 100 ml. ; higher values (8 to 20μg. per 100 ml. or more) are found in thyrotoxicosis, and lower ones (less than 3μg. per 100 ml.) in myxœdema (Rapport and Curtis, 1950). The method is unreliable if iodine has been given to the patient ; moderate amounts may increase the protein-bound iodine and large amounts certainly do so, though it is not the thyroxine content that is affected (Danowski *et al.*, 1950).

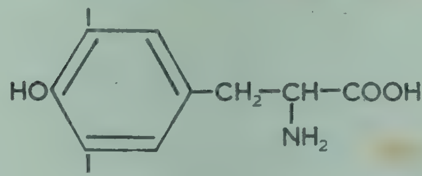
A test of the function of the thyroid gland became available

with the discovery of radio-iodine and is still under investigation. The method consists of measuring the uptake of administered radio-iodine by the thyroid gland which is a measure of the gland's functional efficiency. The estimation can be made by means of a Geiger-Müller counter applied to the neck, or by determining the radio-iodine content of the blood or its elimination in the urine. In thyrotoxicosis the accumulation of the given dose in the thyroid and its disappearance from the blood are more rapid than in normal persons, while in myxœdema the disappearance from the blood is slower and the amount voided in the urine is greater than normal (Pochin, 1950). This method can also be applied to determine the response of the thyroid to injected thyrotropin (Stanley and Astwood, 1949).

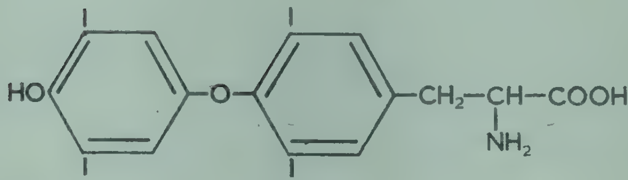
Secondary and somewhat empirical measurements have some use in cases of difficult diagnosis. These are the determination of the blood cholesterol or of creatine tolerance. Blood cholesterol is usually high in myxœdema and low in thyrotoxicosis. Thyrotoxic patients fail to convert creatine to creatinine efficiently ; abnormal creatinuria is often present and subnormal amounts of administered creatine are retained in a tolerance test ; no abnormalities are found in myxœdematous patients.

The question whether hyperthyroidism originates in the pituitary or in the thyroid should be answered by thyrotropin assays made in urine and blood. High values in myxœdema and low ones in thyrotoxicosis have been reported (Purves and Griesbach, 1949). A micro-assay method, depending on the formation of colloid droplets within the thyroid cells, has shown that the thyrotropin in the blood is low in normal thyrotoxicosis and high in cases with malignant exophthalmos (de Robertis, 1948). These methods, however, are still experimental.

Thyroxine Synthesis and Release. The method by which the thyroid gland maintains a concentration of iodide sometimes many hundred times greater than that in the serum is obscure ; apparently most of the iodide in the gland is kept in some peculiar colloidal binding that will not permeate the cell membranes and so is in effect removed from the normal ionic equilibrium mechanisms. The incorporation of the iodide into thyroxine is accomplished in three stages. The iodide has first to be oxidized to elemental iodine ; this then reacts with tyrosine to form di-iodotyrosine, two molecules of which unite by oxidative condensation to form thyroxine. All three stages seem to be enzymically controlled.



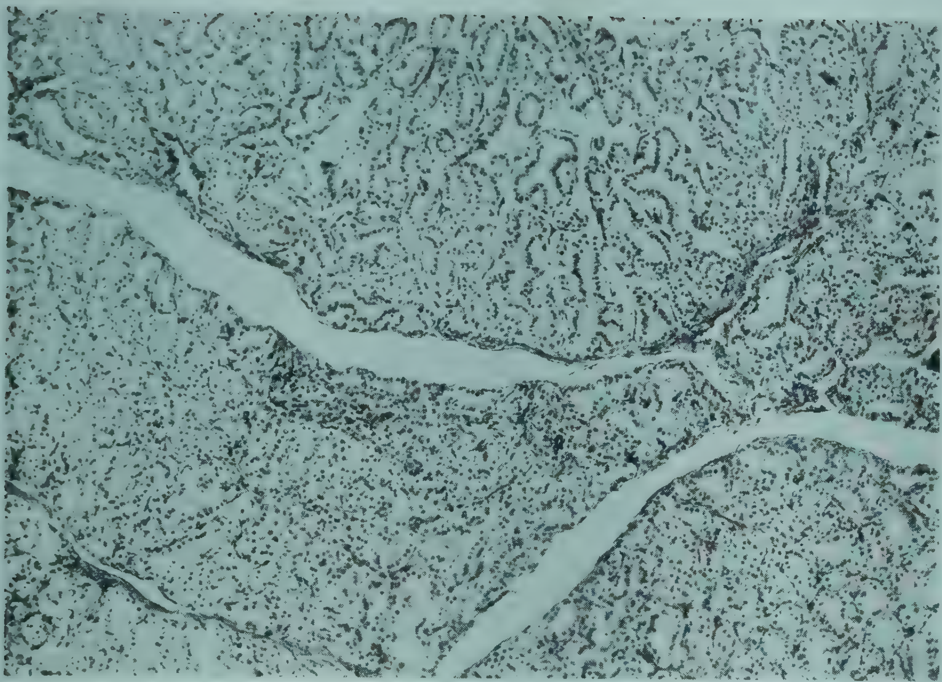
Di-iodo-tyrosine.



Thyroxine.

Thyroxine is stored in the follicular colloid by incorporation in its main component—thyroglobulin. This is a large protein molecule (molecular weight about 700,000) with a variable composition though it contains a fairly constant proportion of tyrosine and/or its iodinated derivatives. Before thyroidal activity can be released into the blood, thyroglobulin is broken down by the action of a proteolytic enzyme (de Robertis, 1941a). This proteolytic activity of the thyroid is increased in thyrotoxicosis or by thyrotropin injection (de Robertis, 1941b). The breakdown products pass into the blood and are incorporated into the plasma proteins. Thyroxine itself when injected shows a long latent period before exerting its metabolic effects ; this and other evidence suggests that it is only active when combined with some form of protein carrier. Casein and other proteins containing tyrosine can be fairly easily iodinated *in vitro* to produce artificial thyroproteins which have thyroidal activity and have been used in place of the natural thyroid hormone in veterinary practice.

Antithyroid Drugs. Paradoxically, the first-known drug to moderate thyroid over-activity was iodine. When a thyrotoxic patient is given iodide in large doses the basal metabolism is reduced, the thyroxine in the blood decreases, and that in the thyroid increases owing to the renewed production and storage of follicular colloid. The reduction in metabolic rate, however, is usually only temporary and it begins to increase again slowly after a time unless the hyperthyroidism is very mild. Iodide treatment was a useful preparation for operation since the lowering of the basal metabolic rate enabled the patient to withstand surgery much better and the decreased vascularity of the gland aided the surgeon's task. The accumulation of thyroxine in the gland,



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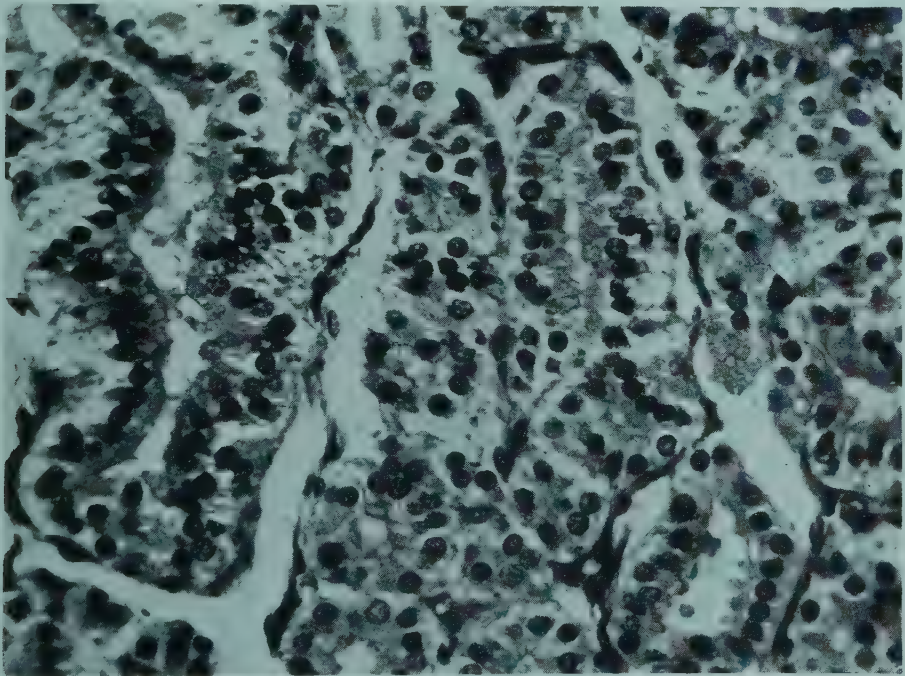

B

Fig. 12/1. Thyroid hyperplasia following prolonged treatment by thiouracil.

(*Above*) Low power. Vesicles are small, closely packed, contain no colloid and have a much reduced lumen. × 70.

(*Below*) High power. There is epithelial activity and elongation of cell axes. × 350.

[*To face page* 352.

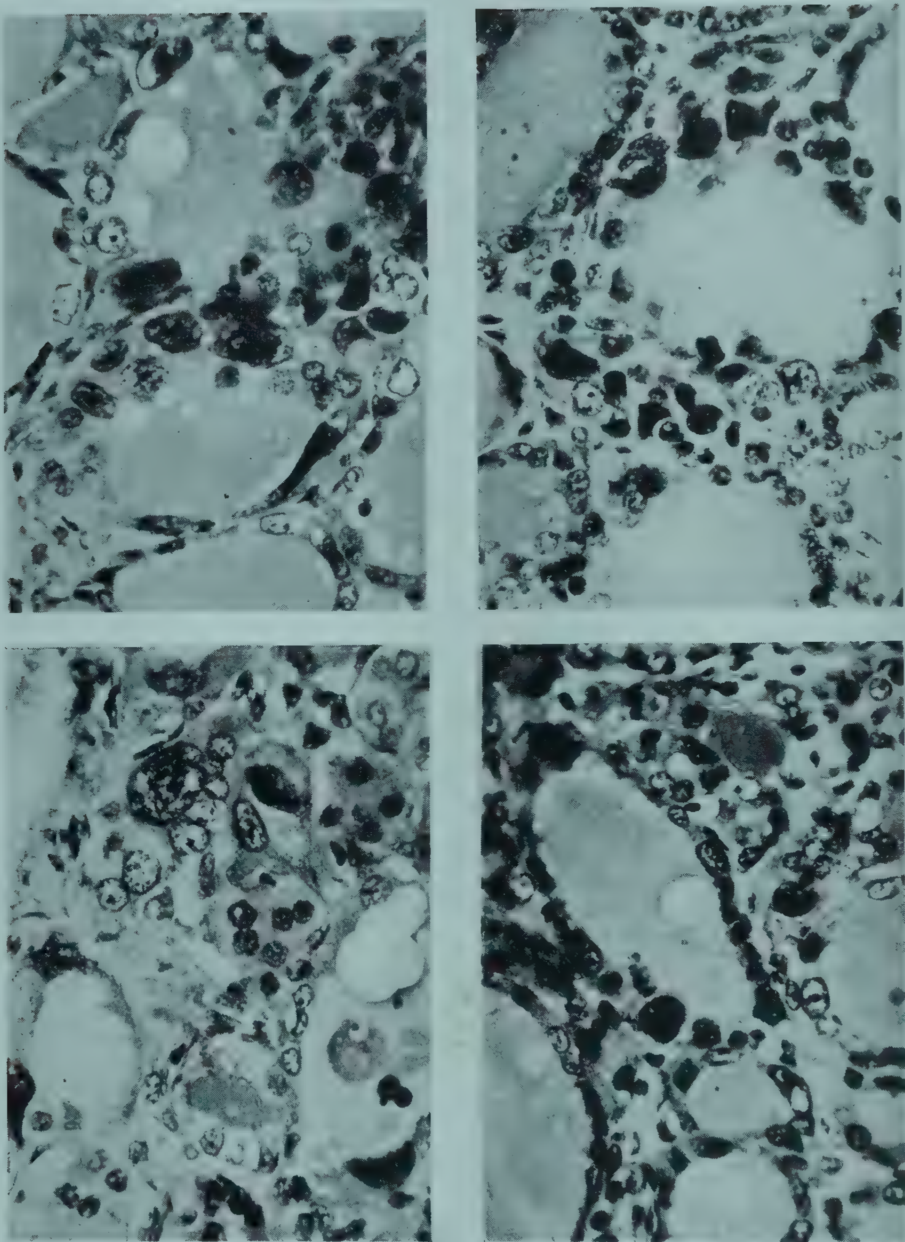

FIG. 12/2. Thyroid hyperplasia following prolonged treatment by thiouracil.

The nuclei of the epithelial cells lining the vesicles show a striking degree of activity. × 350.

however, may be released during operation with ill effects on the post-operative course. The means by which iodine prevents the secretion of thyroxine are complex but inactivation of thyrotropin and of the proteolytic enzyme responsible for the breakdown of thyroglobulin are probably concerned ; iodine has both these actions *in vitro* (Albert *et al.*, 1946 ; de Robertis and Nowinski, 1946).

During the last decade a large number of sulphur-containing drugs have been found to have anti-thyroid activity. They produce goitres when fed to laboratory animals because they prevent thyroxine synthesis in the thyroid gland. The reduction in circulating thyroxine causes increased thyrotropin secretion, hyperplasia of the thyroid, and goitre formation. The most active of these compounds which have been used clinically are thiouracil and its 6-methyl and 6-propyl derivatives. When given to thyrotoxic patients these compounds reduce the basal metabolic rate and the level of thyroxine both in the blood and in the thyroid. Normal metabolism is restored within a few weeks and in most cases can be maintained so indefinitely whilst therapy is continued. Further, when treatment is discontinued after an adequate period (usually eight to twelve months), the metabolism may remain normal and the thyrotoxicosis be cured. (It is as yet too early to assess the proportion of cures with treatment by these compounds, but some of the earliest treated cases show no recurrence after five years.) As a pre-operative treatment thiouracil has the disadvantage that it causes hyperplasia of the gland which becomes very vascular and friable (see Figs. 12/1 and 12/2), but if iodide treatment is combined with thiouracil treatment each neutralizes the disadvantages of the other and the operation can proceed on a patient with normal metabolic rate and a thyroid gland that is relatively avascular and contains little thyroxine. Thiouracil and its derivatives are not without toxic effects, the most dangerous is agranulocytosis which may prove to be fatal. The ideal antithyroid drug has still to be discovered—the early choice of drugs was based on animal experiment and their capacity to produce goitres or to deplete the thyroidal thyroxine or inhibit thyroidal iodine uptake in rats. The results of these tests were not always paralleled by clinical experience in thyrotoxicosis and tests on human subjects have been developed. The relative activities of the drugs in inhibiting the uptake of radio-iodine by the thyroid in man accorded much better with the clinical results (Stanley and Astwood, 1949). Under these conditions 2-mercapto-imidazole

was found to be much more active than any of the thiouracil derivatives, but so far only preliminary reports of the clinical use of this drug have appeared.

The mechanism of action of thiouracil compounds is as complex as that of iodine, though inhibition of the enzyme system necessary for the iodination of tyrosine and reactivation of thyrotropin (the last has been observed *in vitro*) are probably concerned. A third group of antithyroid drugs, of which thiocyanate is the chief example, apparently interferes with the enzyme system controlling the oxidative fixation of iodide in the thyroid gland. Administration of thiocyanate prevents iodide accumulation in the thyroid gland and may even cause the release of that already there.

Iodine Isotopes. Three isotopes of iodine have been used clinically and experimentally. They are I^{128}, I^{130} and I^{131} with half-lives of twenty-five minutes, twelve hours and eight days; they are quoted in the order in which they became available, and the third is now the most commonly used. It emits β-rays chiefly and these have a maximum range of 1 to 2 mm. The isotopes behave chemically exactly like ordinary iodine and so are rapidly concentrated and undergo all the normal transformations of the iodide ion in the thyroid gland. They are, therefore, useful tools in investigations and some of their uses in diagnostic tests have already been mentioned. They are also useful in the treatment of thyrotoxicosis where they have the advantage of applying and localizing radiation to the exact site where it is needed. The hyperplastic gland rapidly concentrates the isotope and its radiation produces degeneration and fibrosis. The basal metabolic rate is restored to normal within several weeks and so far recurrence has been rare. One difficulty is to choose the correct dose; overdosage, like removal of too much of the gland by surgery, produces myxœdema. The theoretical dangers are that the isotope while present in the circulation may damage the germ cells and that the local radiation may induce pre-cancerous changes in the thyroid gland. The rapid accumulation in the thyroid would militate against the first danger, while the limited life of the isotope in the gland (97 per cent. of the activity of I^{131} being emitted in thirty days) would minimize the second danger; there is so far no evidence that either danger has been realized in practice. Isotope therapy might have been expected to be particularly valuable in thyroid cancer, but, unfortunately, most malignant tumours of the gland and their metastases have little or no ability to concentrate iodine. However, functional tumours have been successfully

treated and methods to increase the proportion of suitable cases have been proposed. For instance, the ability to accumulate the isotope in the tumours and/or in remote metastases is sometimes increased by treatment which would render more thyrotropin available to them ; that is, by thyroidectomy, or by thiouracil or thyrotropic injection. The isotope may also be useful in locating metastases (Seidlin *et al*, 1949).

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THE PANCREAS AND DIABETES MELLITUS

Function of the Pancreatic Islets. The implication of the pancreas in regulating carbohydrate metabolism was proved when its removal produced diabetes mellitus in dogs. Since ligature of the pancreatic duct caused atrophy of the acinar tissue but did not affect the islet tissue nor produce diabetes, it was logical to assume that it was the islet tissue that was concerned. This was proved when insulin was isolated from pancreas in which the acinar tissue had been made to atrophy in this way. The failure of previous attempts was due to the proteolytic activity necessarily present in extracts from normal pancreas. Histological evidence suggested that, of the two main types of cells in the islets, it was the β-cells that were the source of insulin. They frequently showed degeneration in cases of diabetes and the insulin content of the pancreas is roughly correlated with their granularity. The most convincing evidence of their function was the discovery that alloxan, a purine derivative closely allied to uric acid, would specifically produce degenerative lesions of the β-cells and diabetes (Lukens, 1948).

Diabetes Mellitus. The chief symptoms of diabetes mellitus are hyperglycæmia, ketosis, increased urinary non-protein nitrogen,

and probably depletion of the liver and muscle glycogen stores. The hyperglycæmia causes glycosuria which in turn necessitates some degree of polyuria. The ketosis leads to the production of ketonic acids which require neutralization by base before they are excreted by the kidneys ; if this drain of base is continued it leads to acidosis, lowering of the blood potassium, and coma. All these symptoms can be corrected by the administration of insulin which, being a protein, has to be given parenterally. Its action is short but can be prolonged by combining it with substances such as protamine and zinc which retard its absorption from the subcutaneous tissues ; the necessity for more than one or two injections daily can be avoided in this way.

Diabetogenic Factors. The absence of insulin is not the only condition necessary for the development of hyperglycæmia and diabetes. This has been completely established by experiments in which removal of the hypophysis (Russell, 1938) or of the adrenal cortex (Long and Lukens, 1936) from pancreatomized animals has abolished or greatly reduced diabetic symptoms. The suggestion that these glands secrete diabetogenic hormones is supported by the opposite experiments in which hyperglycæmia and glycosuria is produced by injection of crude adenohypophysial extracts or of adreno-cortical steroids in large doses (Ingle, 1948). With the adrenal steroids the diabetic symptoms only persist so long as the injections are continued but in some dogs injected with adenohypophysial extracts for some weeks the diabetes may be permanent (Young, 1937).

Functional Tests. The simplest test of the functioning of the carbohydrate-regulating mechanisms of the body is the glucose tolerance test. A normal individual shows a rise in blood-sugar level during the first half-hour after taking a large dose of glucose but no further rise, and usually a fall, during the second half-hour, with recovery of a normal or slightly subnormal blood-sugar level by the end of the second hour. The diabetic receiving the same dose shows a greater rise which is usually continued into the second half-hour period, followed by a slower fall so that the normal levels are usually not regained even by the end of the third hour. This is clear evidence of some failure to deal with excess carbohydrate. The initial dose of glucose stimulates the regulatory mechanisms so that a second dose given after half an hour produces no further rise in normal individuals, but in diabetes and other endocrine disorders the second dose produces a further rise. This test is usually a better index of disturbed function than the one-

dose test. As diabetes can be experimentally produced by other means than destruction or removal of the pancreas in animals, it may be that the failure demonstrated in the glucose tolerance test is not an indication of insulin failure ; and this is in fact the case in Cushing's disease, where the increased production of adreno-cortical steroids is responsible for a glucose tolerance curve resembling that of diabetes mellitus. More information about this aspect of carbohydrate regulation is provided by insulin- or insulin-glucose tolerance tests (Fraser *et al*, 1941). In adrenal insufficiency the fall in blood-sugar level produced by insulin may be greater and the recovery from the induced hypoglycæmia be much slower than normal. The diabetic glucose-tolerance curve in Cushing's disease is distinguishable from that due to insulin deficiency by the fact that it is hardly affected by the simultaneous administration of insulin, though this can abolish the rise in blood-sugar level produced by glucose in normal or diabetic individuals. There is little doubt that the regulation of insulin secretion is directly dependent on the blood-sugar level and little if at all on adenohypophysial or adreno-cortical control. Glucose tolerance is normal in hypophysectomized animals and insulin secretion in the isolated pancreas has been stimulated by increasing the glucose concentration in the perfusing blood (Anderson and Long, 1948).

Action of Insulin. Hyperglycæmia due to insulin lack could be due to increased production of glucose or to a decrease in its use in the body. The diminished glucose tolerance of the diabetic suggests a decreased use and there is other evidence to favour this view. When glucose labelled with radio-carbon is given to normal rats half the radio-activity is in the form of carbon dioxide within six hours ; in alloxan-diabetic rats only 20 per cent. is oxidized in this time and 40 per cent. is excreted in the urine (Zilversmit *et al.*, 1948). The objection to these experiments is that they concern exogenous carbohydrate given to a hyperglycæmic animal. Experiments with eviscerated rats slowly infused with glucose solutions show that glucose does disappear from the blood under these conditions though at a much slower rate than when insulin is added to the infusing fluid (Ingle, 1948). Another approach to the problem is by giving deuterium oxide (heavy water) to animals at such a rate that its ratio to normal water in the body remains constant. Analysis of the various organic compounds in the body at intervals will then show a rise in the proportion of heavy hydrogen they contain and the rate of this rise will be a measure of

the amounts of the compounds that have been synthesized in the intervals. By this method rats have been shown to replace 70 per cent. of their liver glycogen, 20 per cent. of their muscle glycogen, and 40 per cent. of their liver fatty acids each day. The synthesis of liver glycogen in these experiments only accounted for 3 per cent. of the glucose in their diet while fat formation accounted for 33 per cent. of the dietary carbohydrate. In alloxan diabetic rats the conversion to fat was only 5 per cent. of its value in normal rats; glycogen formation still occurred but it seemed to be chiefly from lactic acid and similarly small molecules rather than from glucose (Stetten, 1949). Thus the depletion of body fat in diabetes may be due to decreased formation as well as increased catabolism called into play to provide an alternative source of energy. Ketosis appears as a direct consequence of this increased catabolism. The fasted diabetic continues to excrete glucose; as this is largely derived from protein, untreated diabetics have a highly negative nitrogen balance. It has been claimed that such gluconeogenesis is increased in diabetes but the evidence is not incontrovertible. The more intricate mechanisms by which these various actions are produced are obscure but one is probably revealed by the observation that anterior pituitary extracts will inhibit the action of hexokinase *in vitro* and that this inhibition is prevented by insulin (Colowick *et al.*, 1947). Hexokinase is the enzyme responsible for catalyzing the reaction between glucose and adenosine triphosphate to produce glucose-6-phosphate and adenosine diphosphate. This is the crux of the metabolism of glucose, being the first step both in its conversion to glycogen and in its oxidative degradation to provide energy.

Pancreatic Hyperglycæmic Factor. Study of effects of insulin on liver glycogen is complicated by the presence of an impurity in almost all commercial preparations (including crystalline ones). This substance, which appears to be produced by the α-cells of the islets, for it can be extracted from the pancreas of animals with alloxan diabetes, causes glycogenolysis and an increase in blood-sugar level. It is this factor which presumably accounts for the reduction in the insulin requirements that often follows total pancreatectomy in diabetic patients or in animals. There is some evidence that the factor is present in venous blood leaving the pancreas but this evidence is not yet sufficient to establish the factor as a hormone (Sutherland, 1950).

Cause of Diabetes. The symptoms of diabetes may be produced experimentally in so many ways that it is unlikely that

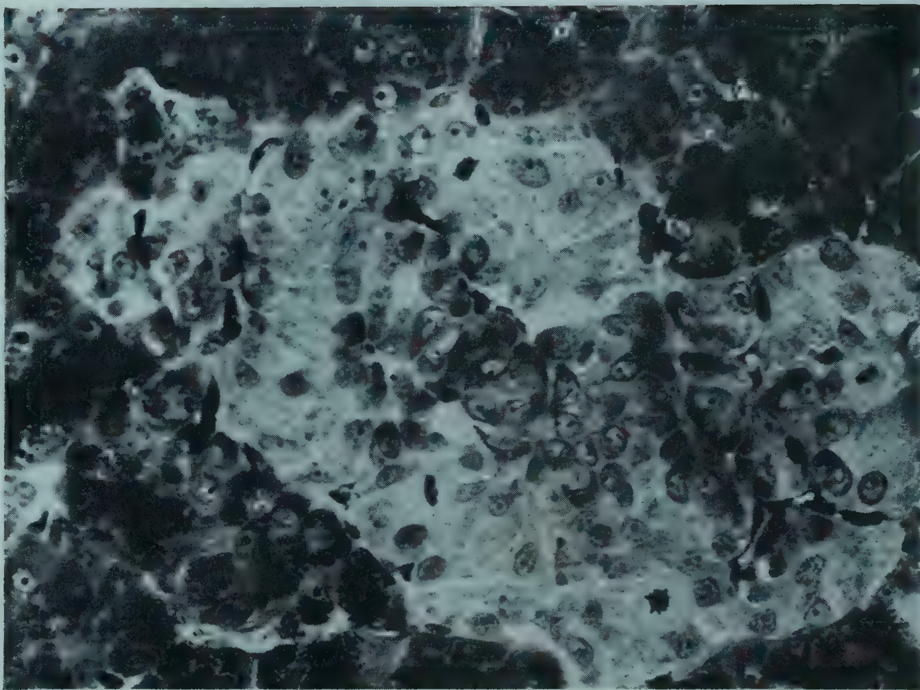

Fig. 12/3.

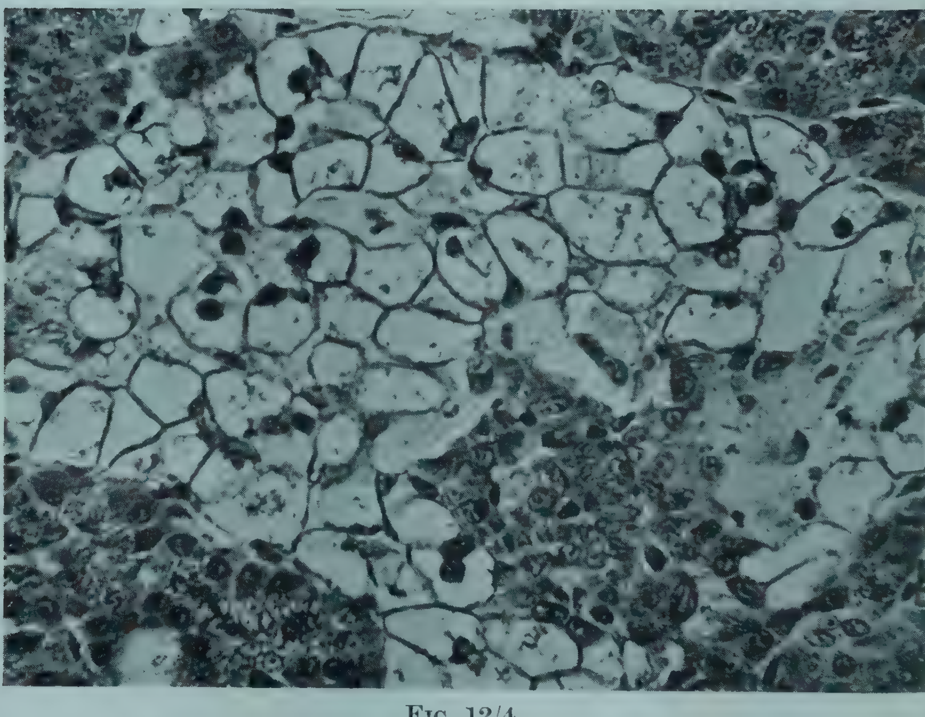

Fig. 12/4.

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all human cases have the same origin. It is even improbable that a failure of insulin secretion is the sole cause of human diabetes. Furthermore, the histological condition of the islets is very variable in human diabetes; the most common accompaniment is "hydropic degeneration" of the β-cells or hyalinization of the islets but no pathological changes can be found in the pancreas of some cases (Figs. 12/3 and 12/4). The term "hydropic degeneration" has proved to be a misnomer, the watery appearance of the swollen cells being caused by glycogen infiltration and the cells will quickly revert to normal in experimental animals when insulin is given (Duff and Toreson, 1951). Two of the experimental methods of producing diabetes are unlikely to be concerned in the human condition. These are the destruction of the islets by alloxan and the disuse atrophy of the islets that may be induced by long-continued insulin treatment (Mirsky *et al.*, 1942). There is, however, circumstantial evidence to connect juvenile diabetes with the diabetogenic activity of the adenohypophysis. Crude hypophysial extracts given to dogs or cats provoke hyperglycæmia and glycosuria (hypophysial diabetes) which in some cases persist after the treatment has been stopped (metahypophysial diabetes). During the initial phase the pancreatic islets show degranulation and some proliferation: glycogen infiltration occurs and this becomes more common and may go on to hyalinization as metahypophysial diabetes supervenes (Richardson and Young, 1938). This permanent condition is not caused by the adenohypophysial extract itself but rather by the hyperglycæmia it produces and the consequent exhaustion atrophy of the islets. If the hyperglycæmia is prevented by giving insulin at the same time as the pituitary extract, then permanent diabetes is not produced (Young, 1944). Collateral evidence for this interpretation is that maintaining hyperglycæmia in a cat for several months by feeding

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FIG. 12/3. Islet of Langerhans from a cat made temporarily diabetic by crude anterior pituitary extract. Note the β cells are partly de-granulated and some are in mitosis. (Preparation stained by phosphotungstic acid and hæmatoxylin.) $\times$ 430.

FIG. 12/4. Glycogen infiltration of β cells from a persistently diabetic cat. Formerly known as "hydropic degeneration," this condition of the β cells is not necessarily indicative of loss of secretory function. It may be reversible or may lead to atrophy of β cells or replacement with hyaline material. (Preparation stained by phosphotungstic acid and hæmatoxylin.) $\times$ 430.

(*Specimens kindly supplied by K. C. Richardson, Esq.*)

sugar has also produced permanent diabetes (Dohan and Lukens, 1948). Adrenocorticotropin, by its action on the adrenal, is only partly responsible for the diabetogenic action of the pituitary extracts ; most of the action is probably due to growth hormone. Extracts which are fully diabetogenic in adult dogs and cats are ineffective in puppies or kittens but the growth of these is greatly increased and signs of acromegaly may be produced ; only when growth ceases is diabetes produced. The onset of most cases of juvenile diabetes coincides with puberty and 86 per cent. of the cases have been shown to be abnormally tall, suggesting that the cause is similar to that in young animals. Excessive growth stimulation will strain the islet tissue but does not cause hyperglycæmia because the protein and carbohydrate which are not used when growth has ceased are readily utilized by the growing organism. It is only when the growth has reached its limit that exhaustion of the islets leads to overt hyperglycæmia (Young, 1949). No one suggests such a mechanism as the explanation of all types of human diabetes. There is little doubt that idiopathic islet failure does occur. Other types of diabetes, particularly those showing resistance to insulin in insulin-glucose tolerance tests, may be due to excess of adenohypophysial or adrenal-cortical diabetogens which antagonize insulin action but do not suppress its production. It is experimentally proved that these diabetogens confer insulin-resistance. Most insulin-resistant diabetics belong to the middle-aged group in which the condition is clearly related to obesity, but the significance of these findings is uncertain.

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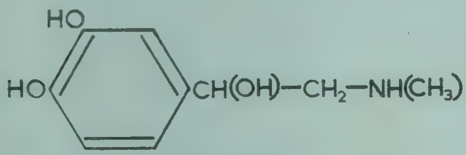
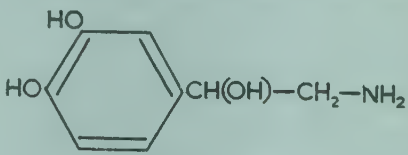
THE ADRENAL MEDULLA

Adrenaline and *nor*-Adrenaline. The discovery that there was more than one pharmacologically active compound in the adrenal medulla was made forty-six years after the isolation of adrenaline. The new factor is called *nor*-adrenaline (the prefix indicating the absence of one radicle, in this case the terminal one) and differs from adrenaline in having no methyl group. This slight chemical difference alters the



Adrenaline.
Methylamino-ethanol-catechol.



nor-Adrenaline.
Amino-ethanol-catechol.

biological activity quite considerably and the difference in action means that all the experimental work done without synthetic adrenaline becomes difficult to interpret, because adrenaline extracted from animal glands (as most commercial preparations are) contains 10 to 20 per cent. of *nor*-adrenaline. The effects of adrenaline in the body vary enormously according to the dosage, the species of animal injected, and the conditions of the experiment. Adrenaline in doses equivalent to those that may be liberated in the body has a general vaso-dilator effect although its cardiac actions whereby the systolic pressure and minute volume are increased may cause an elevation of the blood pressure despite the peripheral vasodilatation. *Nor*-adrenaline, on the other hand, produces widespread peripheral vaso-constriction and has a much greater pressor effect.

Adrenaline is the more active compound in other respects. One of the most important of these is in the short-term regulation of the blood-sugar level. An excess of adrenaline will produce hyperglycæmia or its secretion in response to hypoglycæmia will restore normal blood-sugar level by a coincident breakdown of liver and muscle glycogen. It also stimulates total metabolism as shown by an increase in the oxygen consumption of the intact organism or in the increased metabolism of isolated organs. Its stimulation of adrenal-cortical secretion *via* the adenohypophysis has already been mentioned (p. 326). In all these respects *nor*-adrenaline has only a tenth or less of the activity of adrenaline.

The isolation of *nor*-adrenaline from nerve tissue and its detection in the blood (von Euler, 1951) has probably settled the long-standing problem of the identity of the effector substance liberated at adrenergic nerve-endings. Although these nerves were deemed to be adrenergic because it was presumed that adrenaline was liberated at their endings and mediated their effects, there were many quantitative and qualitative

objections to this view. Early investigators had in fact realized that the properties of *nor*-adrenaline, then only known as a synthetic product of the laboratory, agreed better with those of the neurohumoral transmitter than did those of adrenaline, and it is now clear that sympathetic nerves do liberate *nor*-adrenaline predominantly with only a small proportion of adrenaline. Further investigations may disclose the significance of the different proportions that are liberated by different nerves. This close connection with sympathetic nerve endings and neural transmission, the neural origin of chromaffin cells of the adrenal medulla, and the scattering of these cells in paraganglia throughout the body take adrenaline and *nor*-adrenaline outside the normal ranks of hormones, and makes their study peculiarly difficult. Apart from the obvious difficulty of investigating the normal rate of production from a gland which is so particularly responsive to emotional as well as operative stimuli, production at nerve endings will always make blood estimations of dubious significance. While there is some evidence that adrenaline is secreted from the adrenal medulla in the quiescent animal, there is no doubt that this secretion is enormously multiplied immediately the animal is roused by any emotion or hurt. Most of this rapid response is reflex and mediated *via* the splanchnic nerve from a centre in the vicinity of the fourth ventricle.

Phæochromocytoma. The only pathological change in the adrenal medulla having endocrine repercussions is this tumour affecting the phæochromocytes or chromaffin cells. Such tumours contain large amounts of *nor*-adrenaline and lesser, though still large, amounts of adrenaline. The symptoms are those that might be expected from an over-production of the two compounds: hypertension and some upset in carbohydrate regulation. The hypertension may be sustained or paroxysmal while the blood-sugar level may be raised or normal with a tendency to reduced glucose tolerance. All symptoms disappear if the tumour is successfully removed. The operation may entail great and dangerous outpouring of pressor substance during handling of the tumour and almost equally dangerous hypotension after its removal. Both these dangers can be met by infusing anti-adrenaline drugs during the hypertensive period and *nor*-adrenaline or adrenaline to combat the hypotension. These measures have already considerably reduced the surgical risks. There has been an accelerating spate of reports concerning phæochromocytomas during the past few years, probably as a result of improved methods of diagnosis. Hypertension can be provoked by histamine in those cases where it only occurs paroxysmally, and anti-adrenaline drugs will reduce the pressure in cases which are difficult to differentiate from essential hypertension. The actual mechanism of either test is debated but the results are fairly

constant. Intravenous histamine in cases belonging to the paroxysmal group during a period of normal blood pressure apparently stimulates a release of *nor*-adrenaline and adrenaline from the tumour so that the typical hypertensive spasm is produced. In cases of sustained hypertension the injection of "adrenolytic" drugs such as Dibenamine (N.N.-dibenzyl-β-chloroethylamine hydrochloride) or Piperoxane (933F or piperidyl-methyl-benzodioxane) will produce an immediate lowering of the blood pressure by some mechanism which is certainly not operative in essential hypertension. All these drugs have to be given intravenously and are very potent, and counter-measures for over-dosage have to be at hand. A less drastic test combines the results of pressor tests and postural blood-pressure changes. An even simpler test will probably become available when methods of extracting and assaying urinary *nor*-adrenaline are standardized. As much as 1 mg. of *nor*-adrenaline may be excreted daily in a patient with phæochromocytoma as compared with a normal rate of 30μg. per day (Engel and von Euler, 1950).

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THE PARATHYROID GLANDS

Introduction. The parathyroid glands were not discovered until 1880 and it was soon realized that their removal was responsible for the tetany often produced by thyroidectomy. Parathyroidectomy in rats not only produced tetany but also prevented calcification of the growing teeth. This led to the observation that as a direct consequence of osteomalacia and rickets bilateral compensatory enlargement of the parathyroids took place. It was then erroneously assumed that the parathyroid hyperplasia which accompanies generalized osteitis fibrosa cystica was a consequence of the skeletal disease. The fact that removal of the enlarged parathyroid arrests the skeletal dystrophy constitutes clear and direct proof that the parathyroid hyperplasia is the cause and not a consequence of the bony changes. Meanwhile parathyroidectomy in rats and dogs had been shown to produce a fall in the blood calcium concentration and parathyroid extracts had been prepared which raised the blood calcium level in normal animals and prevented its fall and the accompanying tetany after parathyroidectomy. This was proof that a hormone influencing calcium metabolism was produced by the glands (Albright, 1948).

Calcium and Phosphorus Metabolism. The parathyroid secretion is only one of the factors regulating the economy of these elements in the body. Their concentrations in the blood are influenced by rates of absorption from the gut and excretion in the urine as well as

by their physico-chemical relations within the blood itself and with the massive store of calcium phosphate in the skeleton. Both elements are present in the blood in more than one form. Calcium is combined with protein and is also present as free ions; phosphorus is present in various organic combinations (chiefly in the blood cells) and as ionized inorganic phosphate in the plasma. It is the ionized forms that are influenced by deficiency or excess of parathyroid hormone. The ions form a saturated or even supersaturated system in the blood so that if the concentration of either is increased calcium phosphate tends to be precipitated; when the concentration of either falls, calcium phosphate goes into solution. In practice the blood is protected against the indiscriminate precipitation of the salt by the production of a colloidal form, but if the unbalanced condition persists this protection becomes inadequate and calcification of tissues other than bone will then occur. The equilibrium is normally controlled by the renal thresholds of the elements and by the constant renewal of the skeletal store of calcium phosphate through new bone formation by osteoblast activity and bone dissolution by osteoclast activity. A reduction of the calcium or phosphate concentration in the blood favours bone resorption while an increase in the concentration of either favours bone formation (Schmidt and Greenberg, 1935). The normal level of the serum calcium is 9 to 11 mg. per 100 ml. (of which 4 to 5 mg. is ionized) and the renal threshold for calcium is about 7 mg. per 100 ml. There is therefore normally some calcium in the urine and the amount will be increased if the blood calcium increases or reduced, even to zero, if it falls. Observation of the amount of precipitate formed when a solution of oxalic acid, ammonium oxalate, and acetic acid (Sulkowitch solution) is added to urine samples can therefore be used as a rough estimate of the blood calcium level.

Parathyroid Function. Parathyroidectomy in patients or animals causes phosphate retention with a rise in the blood inorganic phosphate concentration and fall in blood and urinary calcium. Injection of parathyroid extract causes the opposite effects but also produces increased osteoclastic activity with demineralization of bone and its replacement by fibrous tissue. It is argued whether these bone changes are the result of the changes in the blood or *vice versa*. The experimental evidence that the parathyroid secretion directly affects the renal elimination of phosphate is strong. This can conveniently be studied by the injection of phosphate made with isotope-phosphorus. The proportion that is excreted in the urine in rats is greatly reduced by parathyroidectomy but can be quickly increased again by injecting parathyroid extract. When the kidneys are removed parathyroid extract has no effect on the distribution of the labelled phosphate among the tissues (Tweedy *et al.*, 1947). Other experiments have shown that parathyroid extract will no longer cause an increase in blood calcium level after removal of the kidneys (Neufeld and Collip, 1942). This suggests that it is the kidneys rather than the level of labile calcium stored in the skeleton which exercise control. It must, however, be borne in mind that such experiments are not conclusive owing to the great disturbances in the blood produced by nephrectomy. On the other side of the argument there is the evidence that parathyroid

extract will increase osteoclast activity in nephrectomized animals (Selye, 1942). A conservative opinion is that there is positive evidence that parathyroid hormone increases tubular reabsorption of phosphate directly and some evidence that it stimulates osteoclastic activity. There is no reason why the hormone should not have both actions.

Regulation of Function. The parathyroid hormone is a protein that has not yet been purified. It can be assayed by measuring the alterations it produces in the serum-calcium level in dogs or in the inorganic-phosphate level in the blood of rats. No method applicable to biological fluids is established. The glands appear to be amply large for normal requirements and much of the gland tissue can be removed without affecting the function or entailing hypertrophy of the remainder. This does not suggest a gland under adenohypophysial control and no substance having a parathyrotropic action has ever been isolated. The gland enlarges after injection of adenohypophysial extract and is reduced in size after hypophysectomy, but these changes are accompanied by a rise and fall in blood inorganic phosphate respectively (Engfeldt, 1950). It is more likely that the function of the gland is regulated by the chemical composition of the circulating blood than by a pituitary factor ; parathyroid symptoms are not observed in adenohypophysial disorders. Although the phosphate concentration seems to have some effect on the function of the gland, the level of the blood calcium is more potent in this respect. This is proved by experiment in which the two elements are varied independently by dietary means (Stoerck and Carnes, 1945) and it is the lowered blood calcium level that is doubtless responsible for the compensatory hypertrophy of the gland in osteomalacia.

Hypoparathyroidism. Although the condition is sometimes idiopathic it is most commonly the result of removing too much parathyroid tissue during thyroidectomy or parathyroidectomy. The phosphate retention produced causes a rise in blood phosphate and a consequent fall in blood calcium. The urine becomes calcium-free and signs of tetany appear. At first these can only be elicited by clinical tests but gradually localized muscle spasms appear and eventually these increase in severity until general convulsions are produced. Two mechanisms are involved. In the first place there is the increased neuro-muscular irritability responsible for local muscular spasms. Secondly, there are the generalized convulsions apparently due to irritation in the higher nerve centres as they do not occur after section of the spinal cord in animal experiments though the local spasms persist (Carlson and Jacobson, 1911). Meanwhile the precipitation of calcium phosphate leads to an increased density of the bones and to cataracts and other metastatic calcifications. Treatment with parathyroid hormone is not very effective ; it corrects the deficiency temporarily but eventually becomes ineffective. In

animals its administration causes antihormone formation and this is probably the reason for its failure in clinical use. However, there are other effective methods of correcting the calcium-phosphate imbalance. The best is by the administration of dihydrotachysterol (AT10), a product of the irradiation of ergosterol and chemically allied to calciferol but with actions more like that of the parathyroid hormone itself. It not only increases calcium absorption from the gut but also increases phosphate elimination by the kidneys (Albright *et al.*, 1938). Calciferol itself in large doses will restore the blood calcium level by increasing absorption from the gut but still leaves the danger of metastatic calcification as it has no effect on phosphate excretion. The same limitation applies to giving calcium salts in the diet unless measures are taken at the same time to decrease the phosphorus intake (milk being very rich in phosphorus is, for instance, an inadmissible source of calcium).

Hyperparathyroidism. Excessive secretion of parathyroid hormone may be a primary disease caused by hyperplasia or tumour of the gland. It causes a rapid increase in urinary phosphate excretion and a consequent fall in the blood phosphate level. This in turn means that more calcium ions are soluble in the plasma and as the renal threshold for calcium is low the extra calcium constantly drains away in the urine. The increased blood calcium can be maintained by increased calcium absorption from the gut so that bone resorption need not occur, and in fact about half the reported cases of hyperparathyroidism have only slight skeletal symptoms. If the dietary calcium, however, is insufficient to balance the excessive urinary loss then the calcium reserves in the bones have to make good the deficit and generalized osteitis fibrosa is produced. Even if the calcium level is maintained without bone resorption the disequilibrium in the blood entails the same dangers of metastatic calcification as the raised blood phosphate level in hypoparathyroidism, and the formation of renal calculi will eventually aggravate the condition still further. The increased blood-calcium level decreases the neuro-muscular irritability so that a clinical state is produced which is the opposite of tetany and is characterized by hypotonicity and muscular weakness. The only effective treatment of hyperparathyroidism is to reduce the amount of parathyroid tissue or to remove the tumour by surgery. Secondary hyperparathyroidism can be produced in response to a fall in the blood-calcium level or a rise in the blood phosphate level. Such changes may be present in

rickets, osteomalacia, renal insufficiency, pregnancy or dietary calcium deficiency. Compensatory hyperparathyroidism can be distinguished from the primary disorder because the blood-chemical changes tend to be absent or reversed (the blood phosphate level being normal or high and the blood calcium level normal or low) although there is generalized skeletal osteoporosis in both conditions.

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